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TITLE: Cotargeting of Androgen Synthesis and Androgen Receptor Expression as a Novel Treatment for Castration-Resistant Prostate Cancer

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14. ABSTRACT Prostate cancer is the third leading cause of cancer death among American men in 2017. The majority of the death is due to the development of castration resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT). Despite the development and use of next generation anti-AR signaling inhibitors (ASI) such as abiraterone and enzalutamide, resistance to ASI remains the major clinical challenge. The proposed research is based on the finding that protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic activator of AR transcription. If PRMT5 targeting can inhibit or eliminate AR transcription, combining PRMT5 targeting with androgen synthesis inhibition should exhibit a better treatment effect for CRPC. During the past grant period, we have successfully demonstrated that PRMT5 also epigenetically regulates the expression of both AR and AR-V7 in CRPC cells, and that knockdown or inhibition of PRMT5 suppresses growth of CRPC cells. These results support our hypothesis, and we will continue to test our hypothesis during next grant period.											
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1. Introduction

Prostate cancer is the third leading cause of cancer death among American men in 2017 [1], and the vast majority of these patients die of the development of castration resistant prostate cancer (CRPC), a lethal status of the disease [2-4]. The major mechanism underlying the development of CRPC is the reactivation of the androgen receptor (AR), the driver of prostate cancer development and progression. AR reactivation mechanisms include AR overexpression (with or without AR gene amplification), AR mutations, AR splice variants, and androgen-independent activation of AR by AR modulators as well as de novo androgen synthesis in prostate cancer cells [3, 4]. In fact, abiraterone was approved by the FDA in 2011 for CRPC treatment because of its ability to inhibit CYP17A1, a critical enzyme involved in the de novo androgen synthesis in prostate cancer cells [5]. We have recently discovered that protein arginine methyltransferase 5 (PRMT5), an emerging epigenetic enzyme involved in epigenetic control of target gene expression [6-8], is overexpressed in prostate cancer tissues, and its expression positively correlates with the expression of AR [9]. Preliminary data strongly suggest that PRMT5 regulates prostate cancer cell growth through epigenetic control of AR expression. Based on these novel findings, *we hypothesize that co-targeting androgen synthesis and AR expression simultaneously will overcome the mechanisms of AR reactivation and provide an effective treatment for CRPC*. The goal of proposed research is to provide preclinical evidence that inhibiting androgen synthesis by abiraterone in combination with inhibiting or eliminating AR expression by PRMT5 targeting is an effective and novel therapeutic approach for CRPC treatment. We will use CRPC cells and patient derived xenograft (PDX) tumors to test our hypothesis *in vitro* and in mice. Completion of proposed research will provide preclinical evidence to guide the design of future clinical trials (*short-term impact*). If successful, this novel treatment will likely benefit all CRPC patients and ultimately reduce prostate cancer morbidity and mortality (*long-term impact*).

2. Keywords

PRMT5, epigenetics, AR, CRPC, HNPC, ADT, ASI, transcription, abiraterone, enzalutamide

3. Accomplishments

3A. What were the major goals of the project? There are three major goals in this project as defined by three Specific Aims in the approved SOW.

Major Goal 1: To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines

Major Goal 2. To test whether PRMT5 targeting in combination with abiraterone shows a better killing effect in CRPC cells

Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice

3B. What was accomplished under these goals?

Major Goal 1: To determine whether and how PRMT5 regulates the expression of full-length AR and AR splice variants in CRPC cell lines (Months 1-12) Completed.

There are three subtasks in this major goal. The Subtask 1 is to examine the expression of PRMT5, AR, and two major AR variants in CRPC cell lines by RT-PCR, qPCR and Western blotting (Months 1-3), the Subtask 2 is to establish inducible PRMT5 knockdown cell lines and examine the effect of PRMT5 knockdown or inhibition by BLL3.3 and GSK591 on the expression of full-length AR and determine the mechanisms of epigenetic regulation of AR by PRMT5 (months 4-12), and the Subtask 3 is to examine the effect of PRMT5 knockdown or inhibition on the expression of AR splice variants and determine whether the regulation is through the epigenetic mechanism. We have completed this major goal and we summarize our findings as follow.

3B-1. Inhibition of PRMT5 suppresses cell growth and the expression of AR and AR-V7 in CRPC cells.

We established inducible PRMT5 knockdown cell lines in C4-2 cells previously and compared the expression of PRMT5 and AR in C4-2 cells with those in LNCaP cells. We observed a high expression of PRMT5 and AR in C4-2 cells. Importantly, inducible knockdown of PRMT5 suppressed C4-2 cell growth [9]. These results were presented in the original application in Figure 7.

The findings from C4-2 cells led us to hypothesize that PRMT5 may promote CRPC cell growth by epigenetically activating the expression of AR and AR splice variants in general. To test this hypothesis, we proposed to use CWR22Rv1 cells that express AR-V7 [10-12]. To this end, we first treated CWR22Rv1 cells with our inhibitor BLL3.3 (10 μ M) as we did before in LNCaP cells [9] and examined the effect of PRMT5 inhibition on the cell growth using MTT assay. Compared to DMSO control, BLL3.3 significantly inhibited cell growth in a time-dependent manner (Fig. 1A). Next, we examined the effect of PRMT5 inhibition by BLL3.3 on the expression of AR and AR-V7 by Western blotting. As shown in Figure 1B and 1C, BLL3.3 also significantly inhibited the expression of both full-length AR (AR-FL) and AR-V7 by approximately 40%. To determine whether the regulation is through transcriptional regulation, we performed quantitative real time PCR (qRT-PCR) to quantitate the amount of full-length mRNA and AR-V7. Indeed, BLL3.3 also significantly inhibited the expression of both full-length AR and AR-V7 at the mRNA level (Fig. 1D). To verify the regulation of AR and AR-V7 by PRMT5 is through epigenetic regulation, we performed chromatin immunoprecipitation (ChIP) analysis and confirmed that PRMT5 indeed bound to the proximal promoter region of the AR gene (Fig. 1E). Taken together, these results suggest that PRMT5 does regulate the expression of both AR-FL and AR-V7 in CRPC cells.

We also proposed to evaluate the effect of PRMT5 inhibition by the Epizyme inhibitor GSK591 (EPZ015666) [13]. We performed similar MTT assay to determine whether GSK591 can inhibit cell growth in CWR22Rv1. Unfortunately, GSK591 did not show any inhibitor effect (Fig. 2). Consistent with this, treatment of LNCaP cells with GSK591 failed to inhibit cell growth too (Fig. 2). Thus, the Epizyme PRMT5 inhibitor GSK591 (EPZ015666) does not seem to work in prostate cancer cells. In fact, a recent report also suggested that the Epizyme PRMT5 inhibitor

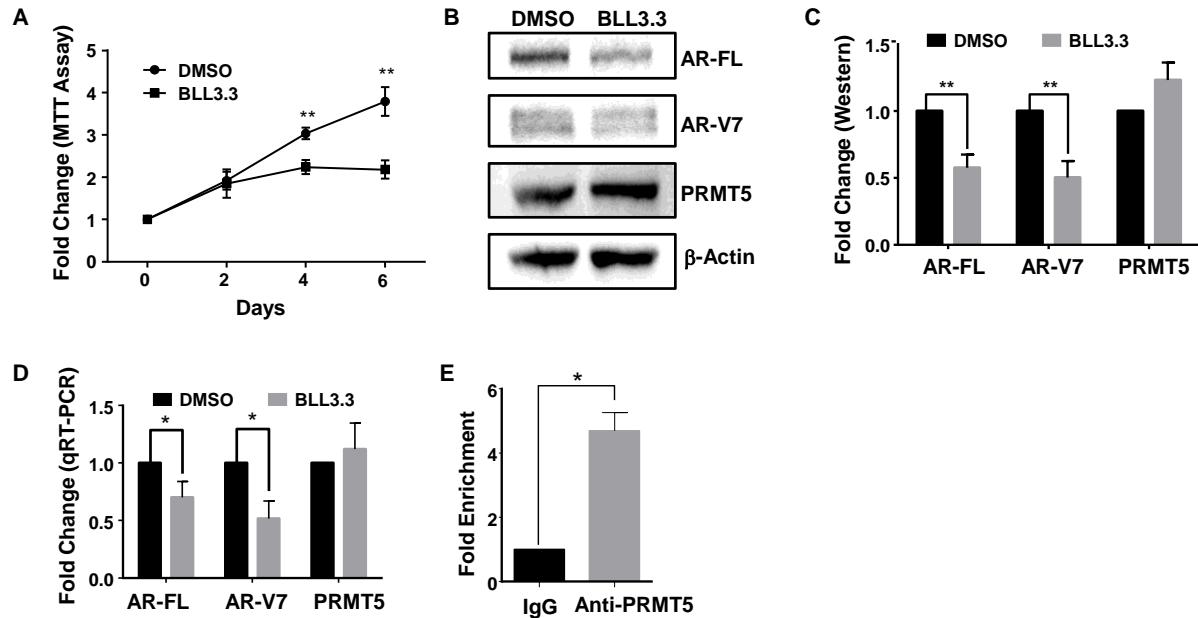


Figure 1. PRMT5 inhibition suppresses CRPC cell growth and down-regulates the expression of AR and AR splice variants. **A.** CWR22Rv1 cells cultured in 48-well plate were treated with 10 μ M of PRMT5 inhibitor BLL3.3 or DMSO control. Cells were incubated for the indicated days and cell viability was analyzed by MTT assay. BLL3.3 was replenished every other day. **B.** CWR22Rv1 cultured in 6 cm dishes were treated with BLL3.3 or DMSO for 6 days and harvested for Western blotting analysis of full-length AR (AR-FL) and AR-V7 as well as PRMT5. β -Actin was used as loading control. Shown is a representative blot from three independent experiments. **C.** The expression of AR-FL and AR-V7 as well as PRMT5 from B was normalized to β -Actin. Shown are mean \pm SD from three independent experiments. **D.** CWR22Rv1 cells cultured in 6 cm dishes were treated with BLL3.3 (10 μ M) or DMSO for 6 days and total RNA was isolated by TRIzol Reagent for determination of mRNA level by quantitative real-time PCR (qRT-PCR). Shown are mean \pm SD from three independent experiments. **E.** Binding of PRMT5 to the proximal promoter region of the AR gene was determined by ChIP analysis followed by quantification with qRT-PCR using anti-PRMT5 antibody or IgG control for immunoprecipitation. Results are mean \pm SD from three independent experiments. * $P<0.05$ and ** $P<0.01$ when Student's *t*-test was used.

failed to inhibit cell growth in MTAP deficient cells whereas knockdown of PRMT5 exhibited a strong inhibition in cell growth [14]. This contradiction was explained by the lack of its access to the target site in PRMT5. In conclusion, the Epizyme inhibitor GSK591 does not work in prostate cancer cells and we will only use our inhibitor BLL3.3 or future improved ones in the proposed work.

As AR-V7 and ARV567ES expression can be induced by abiraterone and enzalutamide in LNCaP95 cells [15], we also proposed to examine whether PRMT5 also regulates the expression of these AR splice variants in LNCaP cells. Contrary to the previous report [15], treatment of cells with either abiraterone or enzalutamide failed to induce the expression of these AR splice variants (Fig. 3). However, PRMT5 inhibition by BLL3.3 also inhibited the expression of AR-FL and AR-

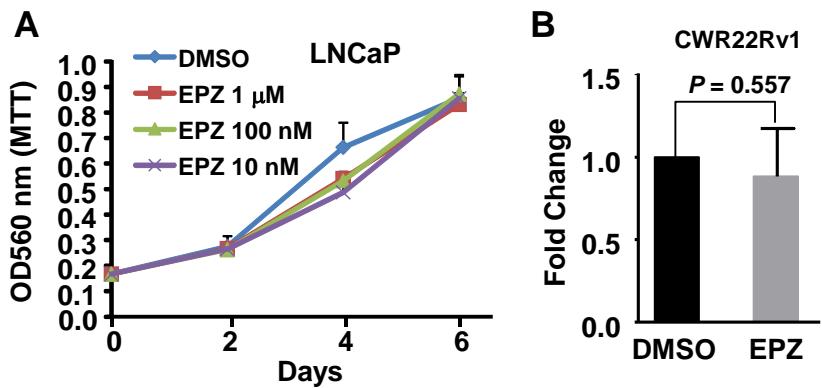


Figure 2. EPZ015666 does not affect cell growth in LNCaP and CWR22Rv1. **A.** LNCaP cells were incubated with various doses of EPZ015666 (EPZ) or DMSO for the indicated days, and assayed for cell proliferation by MTT. **B.** CWR22Rv1 cells were incubated with 10 μ M of EPZ or DMSO for 6 days and fold change in cell growth was determined by Trypan blue. Results in A and B are Mean \pm SD from 3 independent experiments. No statistical significance was found between DMSO and treated groups at each time point (Student's *t*-test). Note that a different experiment with 10 μ M EPZ treatment in LNCaP did not show any growth inhibitory effect either.

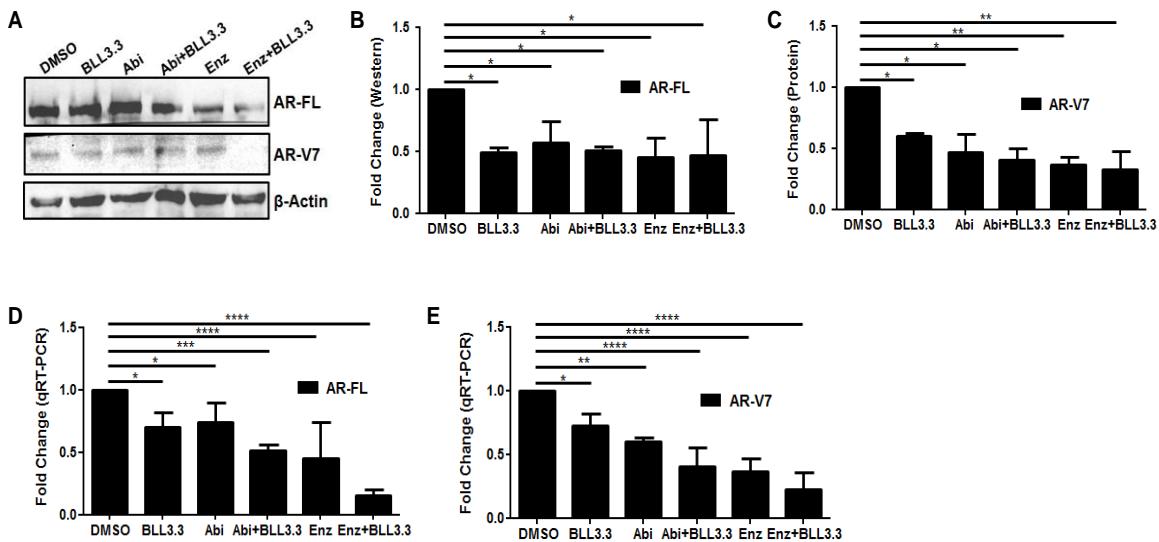


Figure 3. PRMT5 inhibition down-regulates the expression of AR and AR-V7 in LNCaP95. **A.** LNCaP95 cells cultured in 6 cm dishes were treated with BLL3.3, abiraterone (Abi), enzalutamide (ENZ) or DMSO at the final concentration of 10 μ M for 6 days and harvested for Western blotting analysis of full-length AR (AR-FL) and AR-V7. **B** and **C** Quantified results for AR-FL and AR-V7 from A of three independent experiments. **D** and **E**. Similar treatment was performed as A and total RNA was isolated for measurement of mRNA expression of both AR-FL and AR-V7 by qRT-PCR. Shown are mean \pm SD from three independent experiments, and Students *t*-test was performed when compared with DMSO control. * P < 0.05, ** P < 0.01, *** P < 0.001.

V7. Surprisingly, co-treatment of cells with BLL3.3 and enzalutamide exhibited significantly

synergistic down-regulation of both AR-FL and AR-V7, in particular at the mRNA level (Fig. 3D and 3E). We will continue to explore this to see if combining BLL3.3 and enzalutamide or abiraterone exhibit a better treatment effect for the Major Goal 2 during the next grant period.

3B-2. Knockdown of PRMT5 suppresses the expression of AR and AR-V7 and inhibits cell growth in CRPC cells

As discussed above, we observed knockdown of PRMT5 inhibited cell growth and suppressed AR expression in C4-2 cells. Our finding that inhibition of PRMT5 by BLL3.3 also inhibited cell growth and suppressed the expression of both AR-FL and AR-V7 in CWR22Rv1 cells. To corroborate this, we established stable cell lines in CWR22Rv1 using two shRNA constructs identified previously (#1577 and #1836) [9] and examined whether inducible

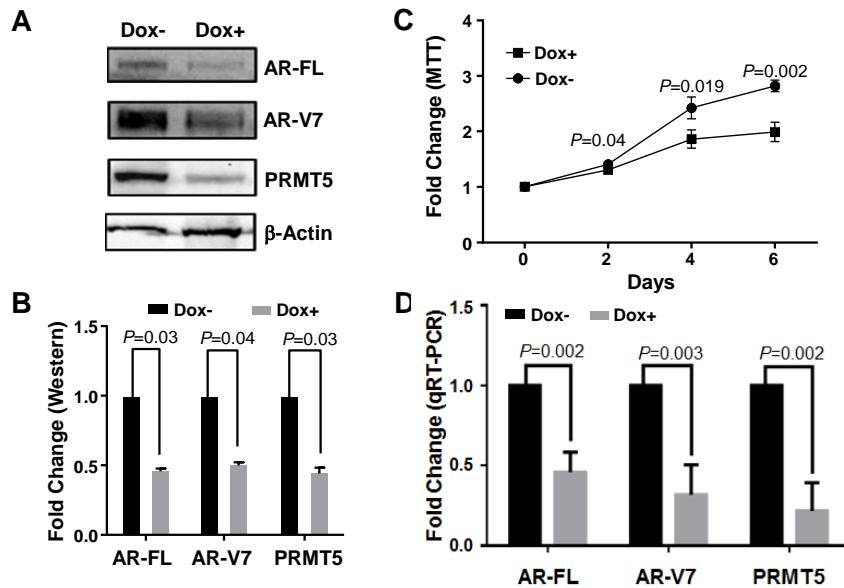


Figure 4. Knockdown of PRMT5 inhibits cell growth and down-regulates the expression of AR and AR-V7 in CWR22Rv1 cells. **A.** Shown are representative results of Western blotting analysis of PRMT5 expression, full-length AR (AR-FL) and AR-V7 in established Doxycycline (Dox)-inducible stable CWR22Rv1-shPRMT5 cells. Cells cultured in 6 cm dishes were induced by Dox (1 μ g/ml) for 6 days and cell lysate was prepared for Western blotting analysis. Dox was replenished every other day. **B.** The protein expression levels in A were quantified by normalizing each protein to β -Actin from three independent experiments. P values were determined by Student's t -test between Dox- and Dox+. **C.** CWR22Rv1-shPRMT5 cells cultured in 48-well plates were similarly treated with Dox at 1 μ g/ml (Dox+) or without Dox treatment (Dox-) for the indicated days and cell viability was assayed by MTT. P values were determined by Student's t -test between Dox- and Dox+ for respective days from three independent experiments. **D.** CWR22Rv1-shPRMT5 cells cultured in 10 cm dishes were similarly treated with Dox for 6 days and total RNA was isolated for qRT-PCR measurement of AR-FL and AR-V7. Note that PRMT5 knockdown was also verified by qRT-PCR. P values were determined by Student's t -test between Dox- and Dox+ for respective days from three independent experiments.

knockdown of PRMT5 can recapitulate the results of BLL3.3. In fact, inducible knockdown of PRMT5 by doxycycline (Dox) inhibited the expression of AR-FL and AR-V7 (Fig. 4A and 4B). Consistent with this, PRMT5 knockdown also inhibited cell growth in a time-dependent manner (Fig. 4C).

As a control, we also established scrambled control (SC) cell line in CWR22Rv1 and induction of PRMT5 knockdown by Dox did not exhibit any effect on cell growth and the expression of AR-FL and AR-V7 (Fig. 5).

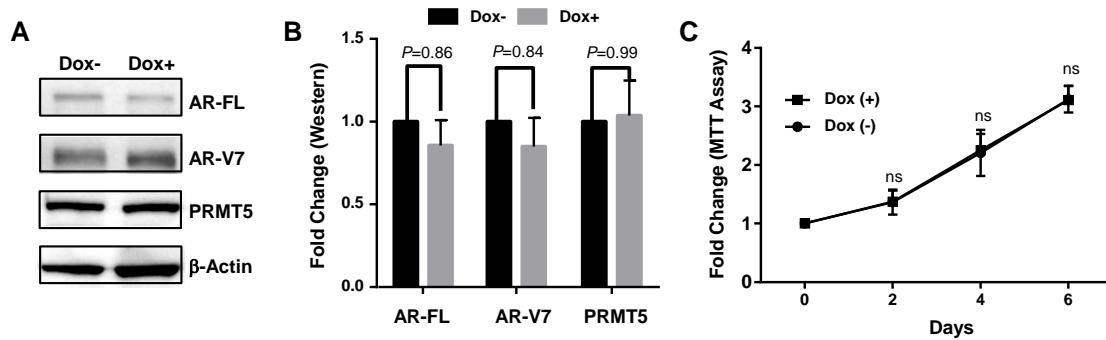


Figure 5. Inducible expression of scrambled control neither affects cell growth nor inhibits AR expression in CWR22Rv1 cells. **A.** Shown are representative results of Western blotting analysis of PRMT5 expression, full-length AR (AR-FL) and AR-V7 in established Doxycycline (Dox)-inducible stable CWR22Rv1-SC cells. Cells cultured in 6 cm dishes were induced by Dox (1 μ g/ml) for 6 days and cell lysate was prepared for Western blotting analysis. Dox was replenished every other day. **B.** The protein expression levels in A were quantified by normalizing each protein to β -Actin from three independent experiments. P values were determined by Student's t -test between Dox- and Dox+. **C.** CWR22Rv1-SC cells cultured in 48-well plates were similarly treated with Dox at 1 μ g/ml (Dox+) or without Dox treatment (Dox-) for the indicated days and cell viability was assayed by MTT. P values were determined by Student's t -test between Dox- and Dox+ for respective days from three independent experiments.

3B-3. Regulation of AR and AR-V7 by PRMT5 is through epigenetic regulation

To rule out the possibility that regulation of AR expression may be through an indirect mechanism (e.g., activation of a transcription factor), we performed luciferase reporter gene (*AR-Luc*) assays in LNCaP cells and found that knockdown of PRMT5 did not inhibit the *AR-Luc* activity. This result suggests that regulation of AR transcription by PRMT5 requires a native chromatin status. Next, we performed chromatin immunoprecipitation (ChIP) analysis and revealed that symmetric dimethylation of H4R3 (H4R3me2s) is highly enriched when compared with H3R8me2s and H2AR3me2s. Importantly, PRMT5 binding to the *AR* promoter region is highly enriched and knockdown of PRMT5 can significantly reduce the binding of PRMT5 to the *AR* promoter region in LNCaP cells. Further, we demonstrated that the transcription factor Sp1 recruits PRMT5 to the *AR* promoter region and the ATP dependent chromatin remodeler Brg1 also participates in the regulation of AR transcription. These results are presented in our recently published Oncogene paper (Figs. 2 and 3), which is attached as Appendix in this report.

To determine whether the epigenetic regulation of AR transcription by PRMT5 in CRPC cells is also via the same mechanism, we performed ChIP analysis for the enrichment of PRMT5, H4R3me2s, Sp1 and Brg1 on the *AR* promoter region, and found that PRMT5 binds to the proximal promoter region of the *AR* gene (Fig. 6A). As a control, PRMT5 does not bind the distant promoter region of the *AR* gene (Fig. 6B). Similarly, the binding of Sp1 and Brg1 to and the enrichment of H4R3me2s at the proximal promoter region of the *AR* gene was also observed. These results demonstrate that PRMT5 regulation of *AR* transcription utilizes the same epigenetic mechanism in both HNPC and CRPC.

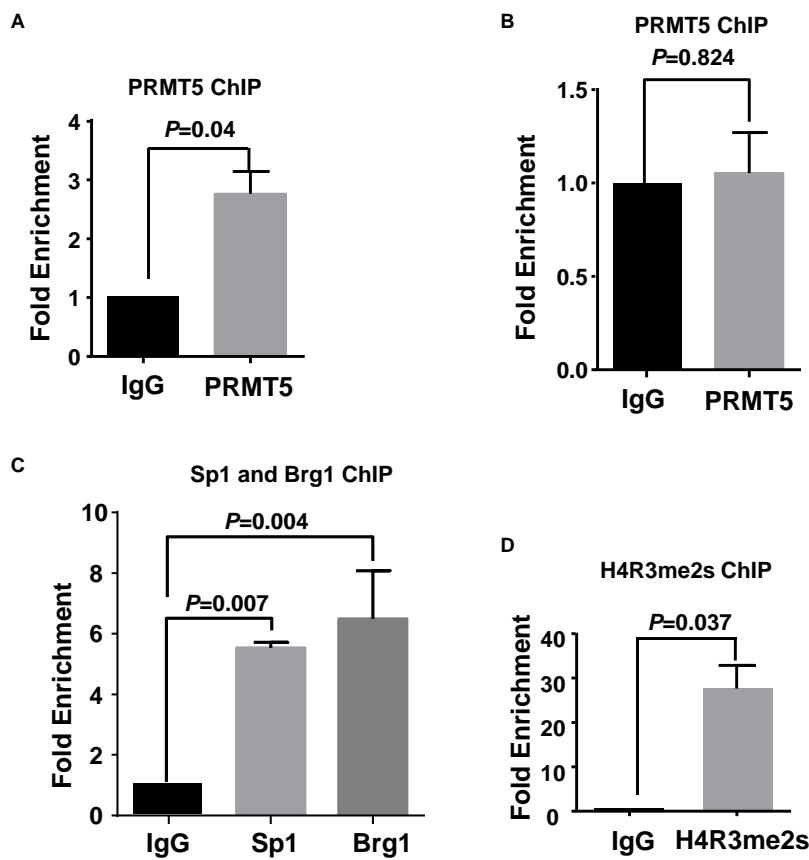


Figure 6. PRMT5 binds to the AR promoter region along with Sp1 and Brg1 and symmetrically methylates H4R3 CWR22Rv1 cells. **A.** Binding of PRMT5 to the proximal promoter region of the *AR* gene was determined by ChIP analysis followed by quantification with qRT-PCR using anti-PRMT5 antibody or IgG control for immunoprecipitation. Results are mean \pm SD from three independent experiments. **B.** No binding of PRMT5 to -5 kb region of the *AR* gene. **C.** Binding of Sp1 and Brg1 to the proximal promoter region of the *AR* gene. **D.** Enrichment of H4R3me2s to the proximal promoter region of the *AR* gene. *P* values were determined by Student's *t*-test between IgG control and the indicated specific immunoprecipitation from at least three independent experiments.

3B-4. PRMT5 is overexpressed in prostate cancer tissues and its nuclear expression correlates with AR expression in prostate cancer tissues

To establish the clinical significance of our findings, we performed immunohistochemistry analysis to measure the expression level of PRMT5 and AR in tissues from benign prostatic hypertrophy (BPH) and prostate cancer. We found that PRMT5 expression in the nucleus correlates with AR expression. Similar correlation at the mRNA levels was also found. These results are presented in Figure 4 in our Oncogene paper (see Appendix).

3B-5. Biological evaluation of a novel PRMT5 inhibitor BLL3.3

As the PRMT5 inhibitor GSK591 from Epizyme did not work in our system, we have been collaborating with Dr. Chenglong Li at Ohio State University (currently University of Florida) to develop a novel type of PRMT5 inhibitors. We have confirmed that the potent PRMT5 inhibitor BLL3.3 is effective and can recapitulate the effect of PRMT5 knockdown in LNCaP cells, DU-145 cells and RWPE-1 cells. These results are presented in Figure 1 and Supplementary Figure S4 in our Oncogene paper (see Appendix) [9].

Major Goal 2. To test whether PRMT5 targeting in combination with abiraterone shows a better killing effect in CRPC cells (Months 13-24)

The finding from LCNaP95 cells (Fig. 3) that BLL3.3 in combination with abiraterone and enzalutamide showed better inhibition on the expression of AR-FL and AR-V7 provides evidence that PRMT5 targeting in combination with abiraterone may be an effective treatment approach. We will conduct the proposed experiments during the next grant period.

Major Goal 3. To evaluate whether PRMT5 targeting plus abiraterone as a combination therapy shows a better treatment effect for CRPC xenograft tumors and patients derived xenografts in mice (Months 1-6 and 19-36)

We submitted documents for PACUC and ACURO and received approvals for the proposed animal experiments (Major Task 3, Subtask 1). We will evaluate whether BLL3.3 or better PRMT5 inhibitors from Dr. Chenglong Li lab can be used for the proposed *in vivo* experiments.

3C. What opportunities for training and professional development has the project provided?

3C-1. Research Trainings. During the grant period, the following four people have been involved in the project and received training (one-on-one research training).

Elena Beketova, a second year graduate student from our PULSe (Purdue University Life Science Umbrella) Program, has been working on the project. Elena was recruited to the lab in May 2016 after she completed one-year rotations. It was a perfect timing for her to work on the project. I spent most of the time during last summer to train her to acquire basic lab skills, in particular molecular biological and biochemical techniques, and she was able to pick up the project relatively independently with the help of the lab technician Xuehong Deng. Over the past year, she has generated most of the data reported in this progress report. She also presented in three meetings and received a Third Place Award for her poster presentation at one meeting (see 3C-2 for details).

Jake Owens, a third year graduate student of MCMP (Medicinal Chemistry and Molecular Pharmacology) program was partially working on the project. His major roles include help for Elena to design and optimize conditions for qRT-PCR and ChIP experiments

Xuehong Deng, a senior lab technician who has been working on the project, continued to work on the project and provided training and technical support to Elena Beketova. She helped generating several stable cell lines that can inducibly express shRNAs to knockdown PRMT5 or MEP50. Importantly, Xuehong completed many experiments on characterization of PRMT5 as an epigenetic activator of AR in prostate cancer cells and she is the first author of the Oncogene paper.

Jonathan Malola, a first year of pharmacy student in the Purdue University College of Pharmacy, has been working on the project under the supervision of Elena Beketova and Xuehong Deng. He has been learning molecular biological techniques and helping with some molecular cloning. In addition, he also tried to use bimolecular fluorescence complementation (BiFC) to investigate the interactions of PRMT5 with MEP50 and several other cofactors (pICln, Riok1 and WDR5). Although he has not generated any conclusive results, he has successfully completed the construction of all BiFC plasmids and it is expected that we will be able to report some results during next grant period.

3C-2. Conference presentations

Elena Beketova, Xuehong Deng, Jake Owens, and Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. 2017 Annual Retreat of Purdue University Center for Cancer Research. Purdue University, October 12, 2016

Elena Beketova, Xuehong Deng, Jake Owens, Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. The Health and Disease: Science, Culture and Policy Research Poster Session, Purdue University, March 23, 2017. Third place award received for poster presentation.

Elena Beketova, Xuehong Deng, Jake Owens, Chang-Deng Hu. Protein Arginine Methyltransferase 5 as an Epigenetic Activator of Androgen Receptor Expression in Castration-Resistant Prostate Cancer. Indiana Basic Urological Research (IBUR) Symposium, June 9, 2017. Oral Presentation

3D. How were the results disseminated to communities of interest?

The Health and Disease: Science, Culture and Policy Research Poster Session was designed to disseminate the discoveries on campus to the entire Purdue community. Elena Beketova presented her major findings on the role of PRMT5 in epigenetic regulation of CPRC in this symposium. The purpose of this symposium is to stimulate the interest in health science and to promote collaborations across the campus.

3E. What do you plan to do during the next reporting period to accomplish the goals?

We have already accomplished the first major goal as we planned. In fact, we even completed more than we proposed to do. An Oncogene paper has been published. As presented in Figure 2, we have confirmed that the GSK591 from Epizyme did not work in our system. As we are collaborating with Dr. Chenglong Li to optimize his PRMT5 inhibitors, we anticipate that we will have a potent inhibitor for proposed experiments in Specific Aims 2 and 3 (Major Goals 2 and 3). Alternatively, we will test other reported PRMT5 inhibitors.

4. Impact

4A. What was the impact on the development of the principal discipline(s) of the project?

Androgen receptor (AR) is the driver of prostate cancer development and progression and is the validated therapeutic target for prostate cancer treatment. Androgen deprivation therapy (ADT) by suppressing androgen levels or inhibiting the activity of AR is the primary treatment option for metastatic disease. Unfortunately, AR reactivation via increased expression (gene amplification), mutation or expression of splice variants that are not responsive to conventional ADT is the underlying mechanisms of resistance to ADT. As such, patients inevitably develop into castration resistant prostate cancer (CRPC). The next generation anti-AR signaling inhibitors (ASI) abiraterone or enzalutamide remain ineffective. The finding that PRMT5 is a novel epigenetic activator of AR transcription represents the first well characterized epigenetic regulator of AR transcription. Thus, targeting PRMT5 could potentially overcome AR reactivation by eliminating AR transcription.

4B. What was the impact on other disciplines?

Although it is generally thought that PRMT5 functions as an epigenetic repressor in multiple human cancers, the current report provides evidence that PRMT5 also functions as an epigenetic activator by symmetrically dimethylating H4R3. This should impact the field of epigenetic regulation by protein arginine methyltransferases.

4C. What was the impact on technology transfer?

Nothing to Report.

4D. What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

Nothing to Report.

6. Products

6A. Publications, conference papers, and presentations

Journal Publications:

Deng, X., Shao, G., Zhang, H.T., Li, C., Zhang, D., Cheng, L., Elzey, B.D., Pili, R., Ratliff, T.L., Huang, J., Hu, C.D. Protein arginine methyltransferase 5 functions as an

epigenetic activator of the androgen receptor to promote prostate cancer cell growth.
Oncogene, 36:1223-1231 (2017)

Presentations by Chang-Deng Hu (PI) not reported above:

06/12/17 Place: Jinan University School of Medicine

Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer

05/15/2017 Place: Northwestern University School of Medicine, Department of Pathology

Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance

7. Participants & Other Collaborating Organizations

7A. *What individuals have worked on the project?*

Name:	Chang-Deng Hu
Project Role:	Hu
Perner ID:	90024721
Nearest person month worked:	1.2
Contribution to Project	Dr. Hu has supervised students and the technician to conduct the proposed research.
Funding Support	Purdue University and PC120512

Name:	Elena Beketova
Project Role:	Graduate Student
Perner ID:	119730
Nearest person month worked:	9
Contribution to Project	Miss Beketova has determined the role of PRMT5 in regulation of AR and AR-V7 expression in CWR22Rv1 and LNCaP95 cells
Funding Support	Department Teaching Assistantship and PC120512

Name:	Jake Owens
Project Role:	Graduate Student
Perner ID:	147536
Nearest person month worked:	3
Contribution to Project	Mr. Owens has helped with qRT-PCR and ChIP analysis
Funding Support	Department Teaching Assistantship and PC120512

Name:	Xuehong Deng
Project Role:	Technician
Perner ID:	90025073
Nearest person month worked:	6
Contribution to Project	Ms. Deng has characterized the role of PRMT5 regulation of prostate cancer cell growth and AR expression in HNPC and CRPC cells and established many stable cell lines. In addition, she has provided training and technical support to students.
Funding Support	PC120512

Name:	Jonathan Malola
Project Role:	Pharmacy Student
Perner ID:	79715
Nearest person month worked:	3
Contribution to Project	Mr. Malola has helped with some plasmid construction
Funding Support	Purdue College of Pharmacy Summer Undergraduate Research Fellowship

7B. Has there be a change in the active other support of the PI/PI(s) or senior/key personnel since the last reporting period?

Previous Active Grants that have been closed during the past grant period.

Title: Generation of PRMT5 transgenic mice for prostate cancer research

Time Commitments: 0.18 Academic year

Agency: Purdue University

Performance Period: 09/01/2016 – 03/31/2017

Level of Funding: \$14,600

Goals: The goal of this cancer center shared resource grant is to use the animal facility to generate a transgenic mouse model to overexpress PRMT5 in prostate.

Role: PI

Overlap: None

Title: Discovery of small molecule inhibitor of PRMT5/MEP50 interaction using Cryo-EM

Time Commitments: 0.18 Academic year

Agency: Purdue University

Performance Period: 12/01/2015 – 5/30/2017 (no cost extension for 6 months)

Level of Funding: \$14,600

Goals: The goal of this internal support from the cancer center is to work with Dr. Wen Jiang to develop a Cryo-EM-based drug discovery method.

Role: PI

Overlap: None

New Active Grants that have been awarded during the past grant period.

Title: Role and targeting of PRMT5 in prostate cancer

Time Commitments: 1.35 Academic Year and 0.45 Summer Months

Agency: National Cancer Institute (1R01CA212403-01A1)

Performance Period: 06/09/2017 – 05/31/2018

Level of Funding: \$547,569

Goals: This is a multi-PI RO1 to investigate how PRMT5 and MEP50 epigenetically reprogram prostate cancer development and progression and to develop a novel type of PRMT5 inhibitors for treatment of both hormone naïve prostate cancer and castration resistant prostate cancer.

Role: Contact PI

Overlap: None

7B. What other organizations were involved as partners?

Nothing to report.

8. Special Reporting Requirements

N/A

9. References

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10. Appendices

10A. Oncogene paper

10B. PI's CV

ORIGINAL ARTICLE

Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth

X Deng¹, G Shao^{1,2}, H-T Zhang^{1,3}, C Li⁴, D Zhang⁵, L Cheng⁶, BD Elzey⁷, R Pili⁸, TL Ratliff^{7,9}, J Huang¹⁰ and C-D Hu^{1,9}

Protein arginine methyltransferase 5 (PRMT5) is an emerging epigenetic enzyme that mainly represses transcription of target genes via symmetric dimethylation of arginine residues on histones H4R3, H3R8 and H2AR3. Accumulating evidence suggests that PRMT5 may function as an oncogene to drive cancer cell growth by epigenetic inactivation of several tumor suppressors. Here, we provide evidence that PRMT5 promotes prostate cancer cell growth by epigenetically activating transcription of the androgen receptor (AR) in prostate cancer cells. Knockdown of PRMT5 or inhibition of PRMT5 by a specific inhibitor reduces the expression of AR and suppresses the growth of multiple AR-positive, but not AR-negative, prostate cancer cells. Significantly, knockdown of PRMT5 in AR-positive LNCaP cells completely suppresses the growth of xenograft tumors in mice. Molecular analysis reveals that PRMT5 binds to the proximal promoter region of the AR gene and contributes mainly to the enriched symmetric dimethylation of H4R3 in the same region. Mechanistically, PRMT5 is recruited to the AR promoter by its interaction with Sp1, the major transcription factor responsible for AR transcription, and forms a complex with Brg1, an ATP-dependent chromatin remodeler, on the proximal promoter region of the AR gene. Furthermore, PRMT5 expression in prostate cancer tissues is significantly higher than that in benign prostatic hyperplasia tissues, and PRMT5 expression correlates positively with AR expression at both the protein and mRNA levels. Taken together, our results identify PRMT5 as a novel epigenetic activator of AR in prostate cancer. Given that inhibiting AR transcriptional activity or androgen synthesis remains the major mechanism of action for most existing anti-androgen agents, our findings also raise an interesting possibility that targeting PRMT5 may represent a novel approach for prostate cancer treatment by eliminating AR expression.

Oncogene (2017) 36, 1223–1231; doi:10.1038/onc.2016.287; published online 22 August 2016

INTRODUCTION

Protein arginine methyltransferase 5 (PRMT5) is a type II arginine methyltransferase that epigenetically regulates gene transcription by symmetrically dimethylating histone H4 arginine 3 (H4R3me2s), histone H3 arginine 8 (H3R8me2s) or histone H2A arginine 3 (H2AR3me2s).^{1,2} PRMT5 also modulates the function of non-histone protein substrates by dimethylating arginine residues on the proteins. By regulating transcription of target genes or post-translational modifications of signaling proteins, PRMT5 is implicated in the regulation of many cellular processes such as cell cycle progression, apoptosis and DNA-damage response. Accumulating evidence shows that PRMT5 is overexpressed in several human cancers, and its expression positively correlates with disease progression and poor outcomes.^{3–8} Mechanistic studies have suggested that PRMT5 may function as an oncogene by epigenetic repression of several tumor suppressor genes or by post-translational modification of signaling molecules.^{9,10}

Prostate cancer remains the most common non-cutaneous cancer among American men.¹¹ Although many molecules and signaling pathways that regulate prostate cancer development

and progression have been identified and characterized, androgen receptor (AR) signaling is the most important factor that drives prostate cancer development and progression.^{12–14} Thus, targeting AR signaling, such as androgen deprivation therapy (ADT), is a standard treatment for patients with locally advanced and metastatic disease. Despite the initial response to ADT, the majority of prostate cancers progress to a lethal status known as castration resistant prostate cancer (CRPC) owing to AR reactivation, which includes AR gene amplification, AR mutations, AR splice variants, and intratumoral *de novo* androgen synthesis in prostate cancer cells.^{13,15,16} Recent evidence further shows that AR reactivation is also the major mechanism of resistance to the two next-generation anti-androgen agents abiraterone and enzalutamide.^{17,18} Therefore, the expression of wild-type or mutant AR is absolutely required in both hormone naive prostate cancer and CRPC. However, compared with extensive studies of AR co-activators and co-repressors including epigenetic regulators,^{19–24} how AR expression is regulated, particularly at the epigenetic level, remains largely unknown.

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Here, we report that PRMT5 is highly expressed in prostate cancer tissues and that its expression positively correlates with the expression of AR. Molecular analysis reveals that PRMT5 epigenetically activates the transcription of AR via symmetric dimethylation of H4R3 and promotes prostate cancer cell growth *in vitro* and xenograft tumor growth in mice. Given that current AR-targeting strategies, which are largely based on the inhibition of AR transcriptional activity or inhibition of androgen synthesis, are ultimately ineffective, our findings raise an interesting possibility that targeting PRMT5 may be explored as a novel therapeutic approach to inhibit or eliminate AR expression for prostate cancer treatment.

RESULTS

PRMT5 expression is required for prostate cancer cell growth in an AR-dependent manner

We and others previously reported that knockdown of PRMT5 inhibited cell growth in LNCaP cells.^{25,26} To further investigate this, we examined the role of PRMT5 in DU145 and PC-3 cells by transiently knocking down PRMT5, and did not observe any significant effect on cell growth when compared with scrambled control (SC; Supplementary Figure S1a-d). Knockdown of PRMT5 in LNCaP cells also exhibited a pronounced inhibitory effect on colony formation in soft agar (Supplementary Figure S1e). Next, we established stable cell lines using LNCaP and DU145 that can be induced by doxycycline (Dox) to express short-hairpin RNA (shRNA), and confirmed that inducible knockdown of PRMT5 indeed showed significant growth inhibition in LNCaP cells (Figure 1a), but not in DU145 cells (Figure 1b). Because DU145 and PC-3 cells do not express detectable level of AR,²⁷ these results suggest that PRMT5 may regulate prostate cancer cell growth in an AR-dependent manner. To confirm this, we established Dox-inducible stable cell lines using LNCaP-derived CRPC cell line C4-2 cells that express a higher level of PRMT5 and AR (Supplementary Figure S2), and normal prostate epithelial RWPE-1 cells that do not express detectable AR in the absence of androgen stimulation.^{28,29} Again, knockdown of PRMT5 significantly inhibited cell growth in C4-2 cells, but had no effect on cell growth in RWPE-1 cells (Figures 1c and d). Consistent with the growth inhibition in LNCaP and C4-2 cells, PRMT5 knockdown also downregulated AR expression (Figure 1e). As a result, the mRNA level of AR target genes *PSA*, *KLK2* and *TPRSS2* was decreased by PRMT5 knockdown³⁰ (Figure 1f). To further confirm that AR mediates the effect of PRMT5 on the regulation of cell growth, we performed a rescue experiment by expressing FLAG-AR under the control of a CMV promoter, and observed that overexpressed FLAG-AR completely abolished the growth inhibition induced by PRMT5 knockdown (Figures 1g and h). Similar results were obtained when the LNCaP stable cell line was used and the target gene expression was partially rescued (Supplementary Figure S3). Thus, AR downregulation is likely responsible for the growth inhibition induced by PRMT5 knockdown.

Recently, a PRMT5-specific small molecule inhibitor Compound 5 (named here as BLL3.3) has been identified.³¹ To determine whether inhibition of PRMT5 by BLL3.3 can recapitulate the effect of PRMT5 knockdown in prostate cancer cells, we treated LNCaP cells with BLL3.3, and observed that the growth of LNCaP cells and the expression of AR were significantly inhibited (Supplementary Figures S4a and b). No inhibitory effect was observed when DU145 and RWPE-1 cells were similarly treated with BLL3.3 (Supplementary Figures S4c and d). These results provide additional evidence that the enzymatic activity of PRMT5 is required for AR expression and cell growth in prostate cancer cells.

AR is an epigenetic target of PRMT5 in prostate cancer cells

To determine how PRMT5 regulates AR expression, we examined the effect of PRMT5 knockdown on AR transcription by performing quantitative real-time PCR (qRT-PCR), and observed that transient knockdown of PRMT5 decreased the mRNA level of AR by ~50% (Figure 2a). As PRMT5 may regulate AR transcription epigenetically or indirectly via the regulation of AR transcriptional regulators, we examined the effect of PRMT5 knockdown on the AR-Luciferase reporter gene (AR-Luc) activity, and observed that PRMT5 knockdown had no impact on the AR-Luc activity (Figure 2b). This result suggests that a native chromatin status is required for the downregulation of AR by PRMT5 knockdown. Thus it is likely through epigenetic control of AR transcription. Indeed, the symmetric dimethylation status of H4R3 was significantly enriched on the proximal promoter region of the AR gene when compared with H3R8 and H2AR3 (Figure 2c), despite that all three antibodies can efficiently immunoprecipitate histones H4, H3 and H2A (Supplementary Figure 5). Knockdown of PRMT5 exhibited a greater inhibitory effect on the methylation status of H4R3 (Figure 2d), but a lesser effect on H3R8 and H2AR3 (Supplementary Figure S6). Consistent with this, knockdown of PRMT5 reduced the binding of PRMT5 to the proximal promoter region of the AR gene (Figure 2e), and decreased the level of H4R3me2s on the AR promoter region (Figure 2f). Further, treatment of LNCaP cells with the PRMT5 inhibitor BLL3.3 also decreased the level of AR and H4R3me2s (Supplementary Figure S4b). Taken together, these results demonstrate that PRMT5 epigenetically activates AR transcription by symmetrically dimethylating H4R3.

PRMT5 interacts with Sp1 and Brg1 on the AR promoter

To determine how PRMT5 is recruited to the AR promoter, we examined whether PRMT5 interacts with Sp1, the major and only well-characterized transcription factor that positively regulates AR transcription in prostate cancer cells.^{32,33} Indeed, Sp1 was co-immunoprecipitated with PRMT5 from LNCaP cells (Figure 3a). Because both H3R8me2s and H4R3me2s are associated with the activation of target gene expression when PRMT5 is associated with the ATP-dependent chromatin-remodeling enzyme Brg1,^{34,35} we performed co-immunoprecipitation and found that Brg1 was also co-immunoprecipitated with PRMT5 from LNCaP cells (Figure 3b). To substantiate this finding, we established a Dox-inducible Sp1 knockdown cell line (LNCaP-shSp1) and confirmed that knockdown of Sp1 indeed repressed AR expression (Figure 3d). Significantly, knockdown of Sp1 in this cell line not only abolished the binding of Sp1 to the proximal promoter region of the AR gene (Figure 3d), but also abolished the binding of PRMT5 (Figure 3e) as well as reduced the binding of Brg1 to the same region (Figure 3f). These results together suggest that Sp1, PRMT5 and Brg1 form a complex on the AR proximal promoter region to activate AR transcription.

PRMT5 is overexpressed in human prostate cancer tissues and correlates with AR expression

Next, we examined the expression level of PRMT5 in a human prostate cancer tissue microarray (TMA) consisting of 32 benign prostatic hyperplasia (BPH) tissues and 40 prostate cancer tissues (20 with Gleason score 6 and 20 with Gleason score ≥ 7), and found that PRMT5 expression was significantly higher in prostate cancer tissues than BPH tissues (Figure 4a). Although there is no statistically significant difference in the expression scores between prostate cancer tissues with Gleason score 6 and those with Gleason score 7 and above, 60% of prostate cancer tissues with Gleason score 7 and above showed moderate to high expression (total expression score 40–60) of PRMT5 whereas 40% of prostate cancer tissues Gleason score 6 had similar expression of PRMT5.

Because PRMT5 subcellular localization appears to be an important determinant of cell fate,^{36,37} we compared the expression level of PRMT5 in both the cytoplasm and the nucleus

and observed that some cells showed more nuclear or cytoplasmic localization of PRMT5. However, there was no significant difference in PRMT5 subcellular localization in either BPH tissues

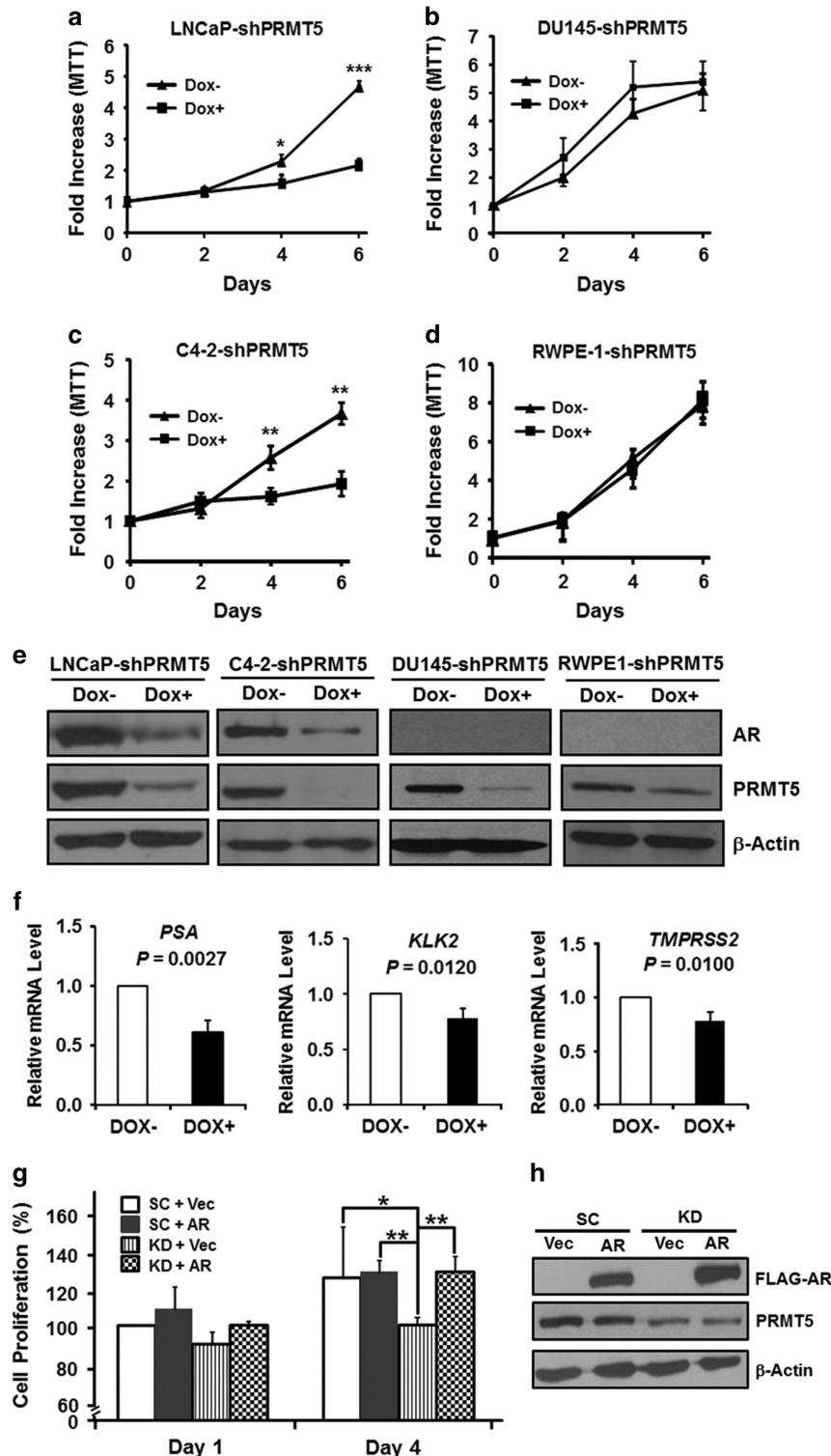


Figure 1. PRMT5 regulates prostate cancer cell growth in an AR-dependent manner. (a-d) Induction of PRMT5 knockdown by doxycycline (Dox+) inhibited cell proliferation in AR-expressing LNCaP and C4-2 cells but not in DU145 and RWPE-1 cells that do not express AR. (e) PRMT5 knockdown induced by Dox decreased AR expression in LNCaP and C4-2 stable cell lines. (f) Knockdown of PRMT5 in LNCaP-shPRMT5 cells reduced the mRNA level of the indicated AR target genes measured by qRT-PCR. (g) Restored cell growth by exogenous expression of FLAG-AR in LNCaP cells transiently co-transfected with SC, or pLKO-Tet-On-shPRMT5 (KD) in combination with pFLAG-CMV (Vec) or pFLAG-CMV-AR (AR). (h) Representative Western blots from g to verify the expression of FLAG-AR and the knockdown of PRMT5. *P < 0.05; **P < 0.01; and ***P < 0.001.

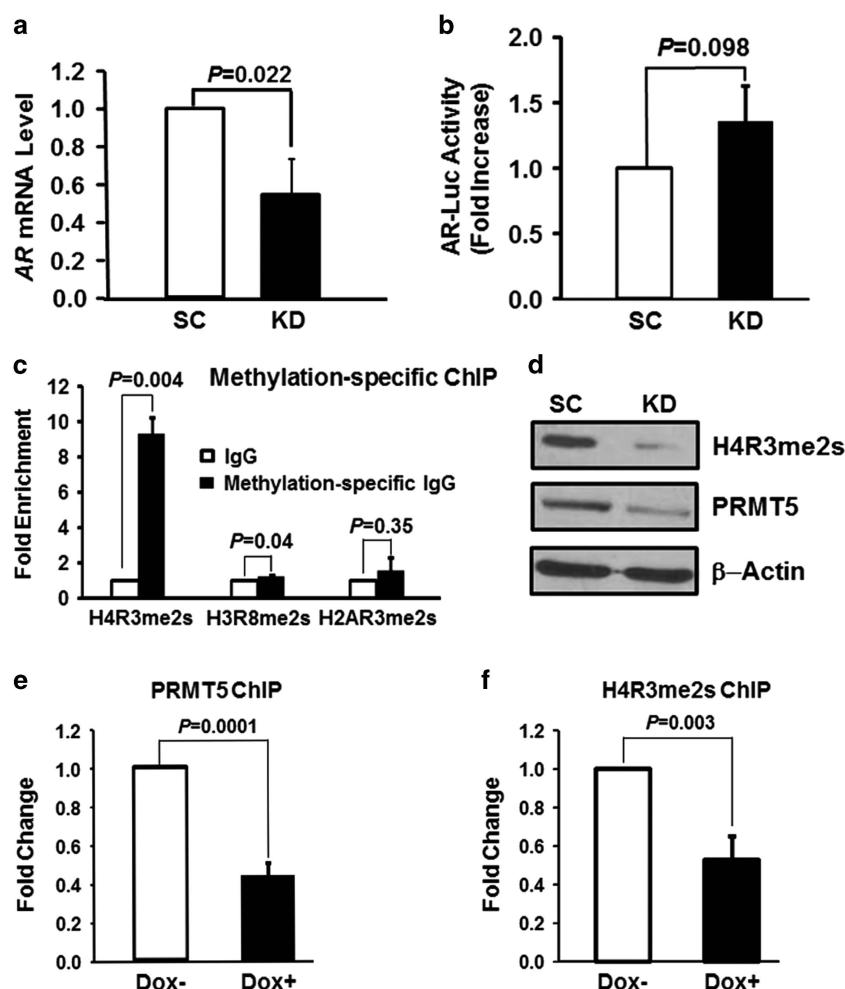


Figure 2. Epigenetic activation of AR transcription by PRMT5 in LNCaP cells. **(a)** Transient knockdown of PRMT5 (KD) reduced AR mRNA level when compared with SC. **(b)** Transient knockdown of PRMT5 had no effect on the AR-luciferase reporter gene (AR-Luc) activity. **(c)** Enrichment of H4R3me2s, but not H3R8me2s and H2AR3me2s, on the proximal promoter region of the AR gene in LNCaP cells. **(d)** Transient knockdown of PRMT5 reduced symmetric dimethylation of H4R3 (H4R3me2s). **(e)** Knockdown of PRMT5 induced by doxycycline (Dox+) reduced PRMT5 binding to the proximal promoter region of the AR gene when compared with cells without Dox (Dox-). **(f)** Knockdown of PRMT5 induced by doxycycline (Dox+) reduced the enrichment of H4R3me2s on the proximal promoter region of the AR gene when compared with cells without Dox (Dox-).

or prostate cancer tissues (Supplementary Figure S7). To analyze the correlation between AR and PRMT5 expression, we examined the expression of AR from the same TMA. In fact, PRMT5 expression in the nucleus correlated positively with AR expression in prostate tissues (Figures 4b and c). We also retrieved data from Oncomine that have >60 cases in each study, and found that PRMT5 expression correlated with AR at the transcript level in prostate cancer tissues (Figure 4d). Thus, it is likely that nuclear-localized PRMT5 may activate AR transcription in prostate tissues.

PRMT5 knockdown inhibits AR expression and suppresses the growth of xenograft tumors in mice

To determine whether PRMT5 expression is necessary for the growth of xenograft tumors in mice, we used Dox-inducible stable cell lines expressing PRMT5 shRNA (LNCaP-shPRMT5) or SC (LNCaP-SC) to establish xenograft tumors in nude mice. As shown in Figure 5a, knockdown of PRMT5 completely suppressed the growth of LNCaP xenograft tumors. In fact, tumor growth in 8 out of 10 Dox-treated mice were completely suppressed. There was no significant difference in the growth of tumors derived from LNCaP-SC regardless of the Dox status (Figure 5b). The expression

level of PRMT5 and AR was also downregulated in Dox-treated residual tumor nodules derived from LNCaP-shPRMT5 when compared with Dox-untreated (Figure 5c). Similar expression of PRMT5 and AR was observed in SC control tumors regardless of the Dox status (Figure 5d). These results demonstrate that PRMT5 is required for the growth of xenograft tumors in mice.

DISCUSSION

AR signaling is a critical determinant of prostate cancer development and progression. Many studies have characterized how AR transcriptional activity is modulated by its co-activators and co-repressors.^{19,21,24} However, how the transcription of AR itself is regulated, particularly at the epigenetic level, remains poorly understood. Here, we provide evidence showing that PRMT5 is a novel epigenetic activator of AR transcription in prostate cancer. First, knockdown of PRMT5 or inhibition of PRMT5 by a small molecule inhibitor specifically inhibited the growth of prostate cancer cells in an AR-dependent manner. Second, knockdown of PRMT5 specifically inhibited AR transcription. Third, PRMT5 binds to the proximal promoter region of the AR gene along with Sp1

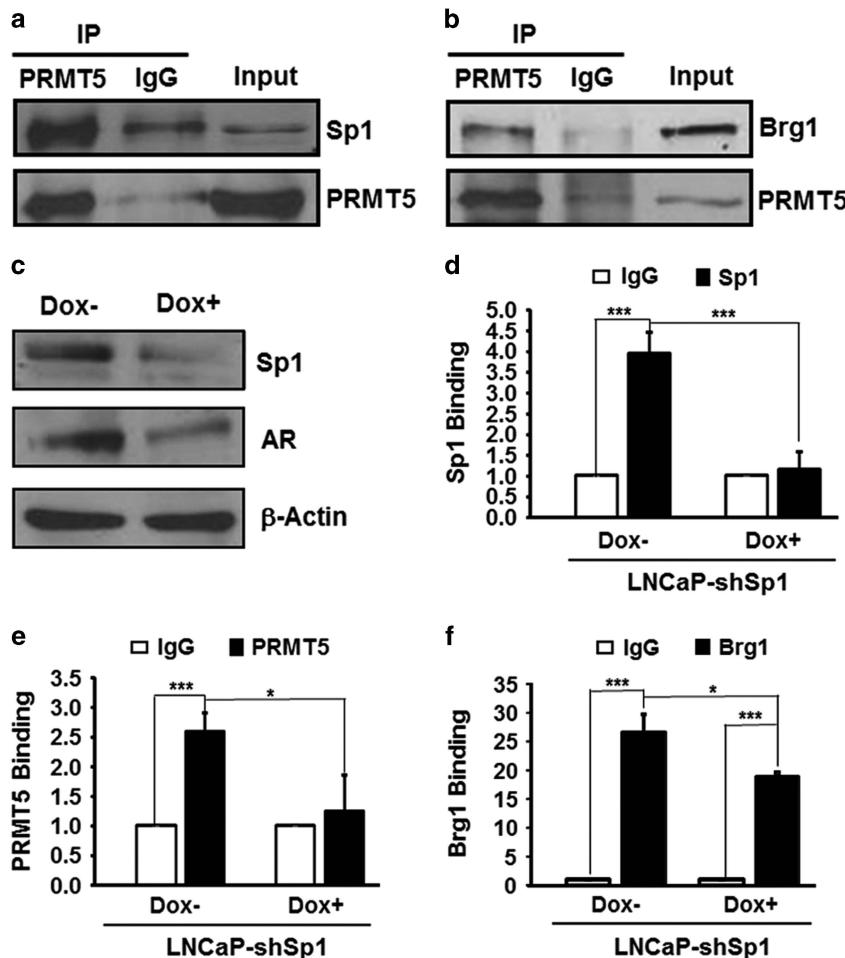


Figure 3. PRMT5 interacts with Sp1 and Brg1 on the proximal promoter region of the AR gene in LNCaP cells. **(a)** Co-immunoprecipitation of Sp1 with PRMT5. **(b)** Co-immunoprecipitation of Brg1 with PRMT5. **(c)** Knockdown of Sp1 induced by doxycycline (Dox+) reduced AR expression in Dox-inducible stable cell line LNCaP-shSp1. **(d-f)** Dox-induced knockdown of Sp1 reduced the binding of Sp1, PRMT5 and Brg1 to the same proximal promoter region of the AR gene. * $P < 0.05$ and *** $P < 0.001$.

and Brg1. Fourth, H4R3me2s is highly enriched on the proximal promoter region of the AR gene. Fifth, PRMT5 is highly expressed in prostate cancer tissues and its expression correlates positively with AR expression at both mRNA and protein levels. Finally, depletion of PRMT5 expression completely suppressed the growth of LNCaP xenograft tumors in mice by downregulating AR expression.

Transcriptional regulation of gene expression is a tightly regulated process that involves the participation of multiple transcriptional regulatory proteins such as transcription factors, co-activators and co-repressors as well as chromatin-remodeling enzymes. Consistent with the fact that Sp1 is the major and well-characterized transcription factor that activates AR transcription in prostate cancer cells,^{33,38} we indeed confirmed that Sp1 binds to the AR promoter and regulates AR expression in LNCaP cells. Because PRMT5 interacts with Sp1 and Brg1 and because Sp1 knockdown also reduces the binding of PRMT5 to the AR promoter, we suggest that Sp1 may recruit PRMT5 to the AR promoter. Interestingly, Brg1, an ATP-dependent chromatin remodeler,³⁹ was also recruited to the AR promoter through its interaction with PRMT5. This finding suggests that PRMT5-mediated H4R3 dimethylation could also activate transcription of target genes such as AR when Brg1 is recruited to the promoters (Figure 6), though PRMT5 generally represses transcription of target genes. Interestingly, PRMT5-mediated H3R8

dimethylation is also involved in transcriptional activation of target genes when Brg1 is recruited to the target gene promoters.^{34,35} Although this manuscript was in preparation, a recent report showed that PRMT5 can dimethylate H4R3 and H3R8 to regulate the expression of the protein kinase FLT3 in acute myeloid leukemia cells via two distinct pathways.⁴⁰ Thus, dimethylation of either H3R8 or H4R3 by PRMT5 may permit ATP-dependent chromatin remodeling, leading to activation or repression of target gene transcription. Given that PRMT5 and Brg1 also cooperate to repress transcription of target genes⁴¹⁻⁴³ and that AR transcription is subjected to the regulation of DNA methylation and histone lysine methylation,⁴⁴ it is likely that AR transcription is subjected to a high order of epigenetic regulation. Future studies to gain insight into the epigenetic regulation of AR may offer new opportunities to develop novel targeting strategies to inhibit or even eliminate AR expression. Because PRMT5 may exhibit an opposite role in the cytoplasm and nucleus in cells,^{9,25} it remains to be determined whether cytoplasmic- and nuclear-localized PRMT5 may have distinct effects on the transcription of AR.

The present finding has significant clinical implications due to the central role of AR in prostate cancer development and progression. Our findings here, together with a previous study showing that PRMT5 may form a complex with MEP50 and AR to modulate the transcriptional activity of AR,⁴⁵ raise an interesting

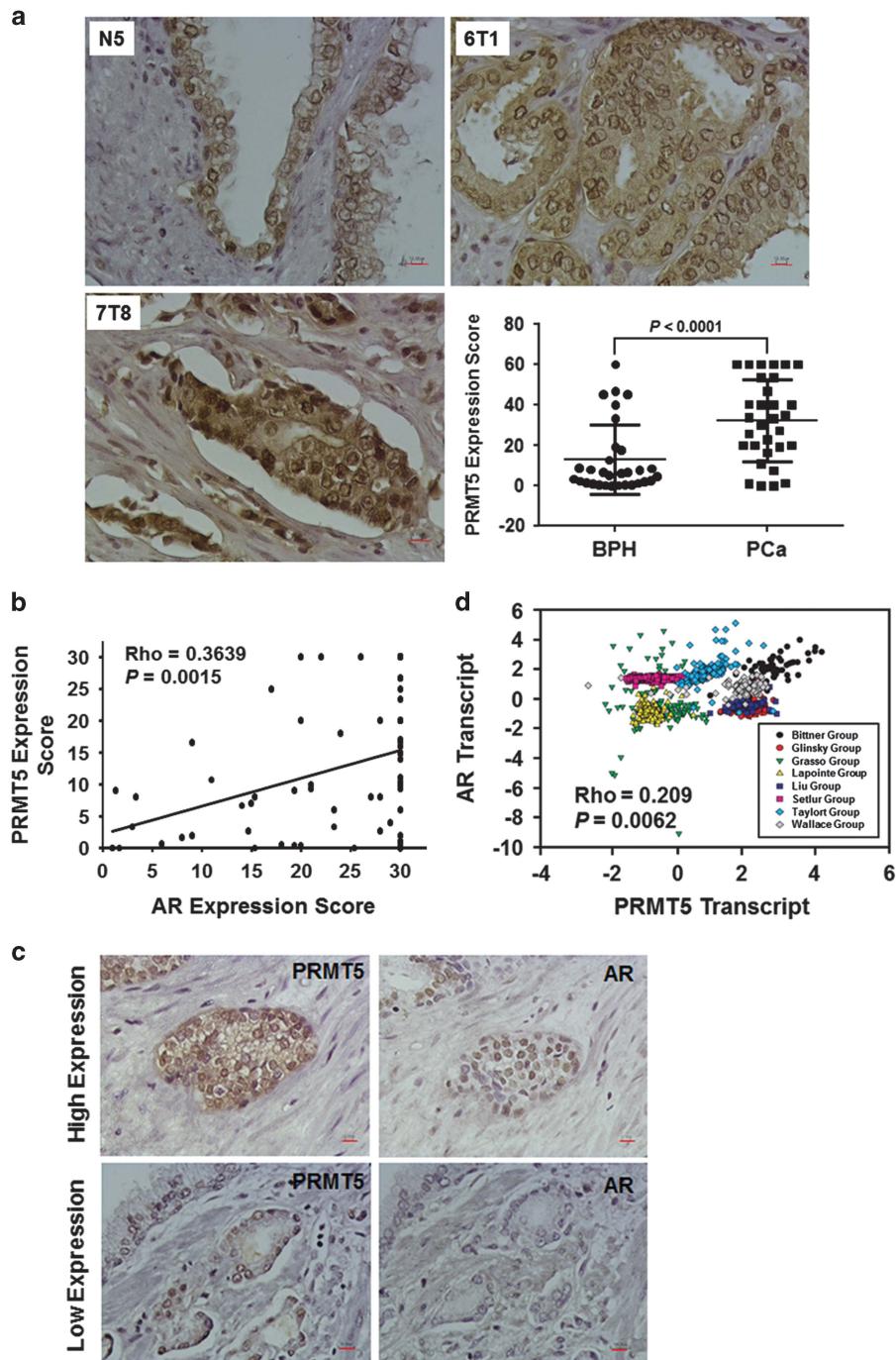


Figure 4. PRMT5 expression correlates positively with AR expression in prostate cancer. **(a)** Shown are representative immunohistochemistry staining images (magnification $\times 400$) of PRMT5 in benign tissue (N5), Gleason 6 prostate cancer tissue (6T1) and Gleason 7 prostate cancer tissue (7T8). The total expression score of PRMT5 is significantly higher in prostate cancer tissues (PCa) when compared with BPH. Scale bar, 30 μ m. **(b)** PRMT5 expression correlates positively with AR expression at the protein level in the same TMA from **a**. **(c)** Representative images of PRMT5 and AR expression from serial sections of prostate cancer tissues. The upper panels show higher expression of both PRMT5 and AR in the nucleus and the lower panels show weaker expression of both PRMT5 and AR in the nucleus. Scale bar, 30 μ m. **(d)** PRMT5 expression correlates positively with AR expression at the transcript level. The data were retrieved from Oncomine database.

possibility that targeting PRMT5 may have a dual effect on both the expression and activity of AR. Thus, PRMT5 may be an ideal target for development of novel therapeutics. As radiotherapy in combination with adjuvant ADT is the current standard treatment for locally advanced prostate cancer, combining radiotherapy with PRMT5 targeting may be an alternative approach. Perhaps

targeting AR expression by inhibiting PRMT5 may avoid some adverse effects often seen with ADT. It is worth noting that PRMT5 also regulates the expression of AR in the CRPC line C4-2. As AR reactivation is the major mechanism underlying the development of CRPC^{13,14} and the resistance to the next-generation anti-androgen therapy,^{17,18} targeting PRMT5 alone or in combination

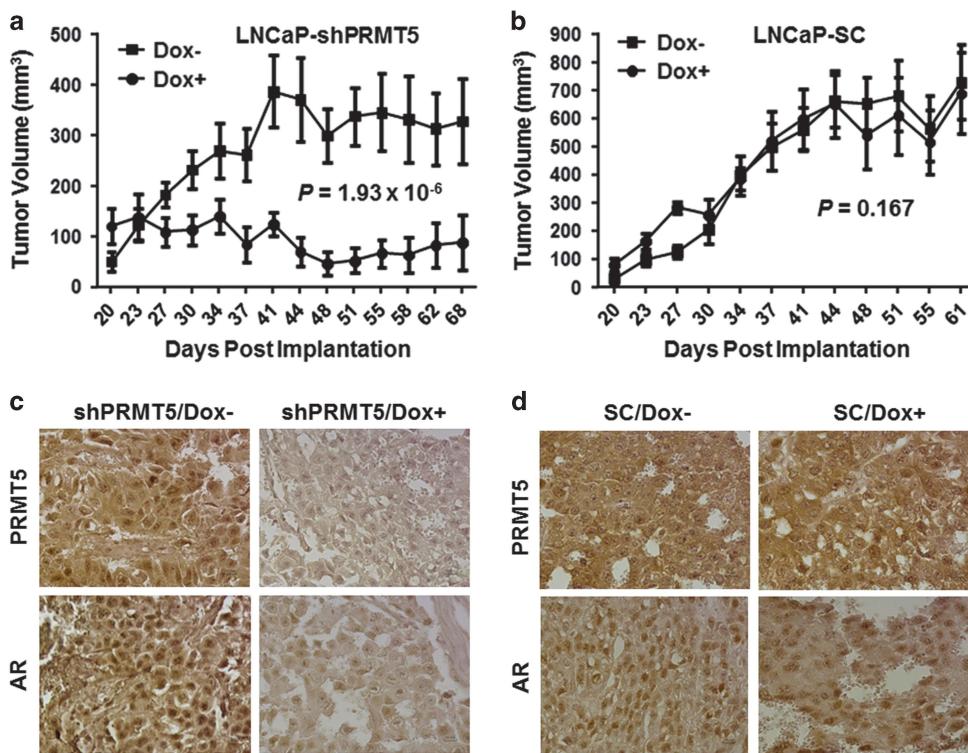


Figure 5. Knockdown of PRMT5 suppresses the growth of xenograft tumors in mice. (a) LNCaP-shPRMT5 cells were implanted subcutaneously into the right lower flanks of 10 nude mice per group, and the tumor growth was monitored twice weekly in Dox-treated (Dox+) and untreated (Dox-) mice. (b) Similar experiment was performed as described in a for LNCaP-SC cell line. (c and d) Representative images showing inhibition of PRMT5 and AR expression in Dox-treated tumor nodules. No effect on PRMT5 and AR expression in xenograft tumors derived from LNCaP-SC was observed. Scale bar: 10 μm.



Figure 6. Proposed model for epigenetic activation of AR transcription by PRMT5.

with other AR-targeting agents may exhibit a better treatment efficacy than the existing treatments. Given that two small molecule inhibitors of PRMT5 have been developed,^{31,40,46} preclinical evaluation of these inhibitors alone or in combination with radiotherapy or other AR-targeting agents may lead to the development of novel therapeutic approaches for prostate cancer treatment.

MATERIALS AND METHODS

Cell lines and culture

Prostate cancer cell lines LNCaP, DU145, and PC-3 as well as RWPE-1 cells were purchased from ATCC (Manassas, VA, USA) and C4-2 cells were purchased from M.D. Anderson Cancer Center (Houston, TX, USA). All frozen stock received were immediately expanded and aliquots were prepared and stored in liquid nitrogen for future use, and cells were maintained for no longer than 3 months as described previously.^{26,47} Cell line authentication was performed by IDEXX BioResearch (IMPACT I). The establishment of stable cell lines was described previously.

Plasmid construction

The pLKO-Tet-On plasmid for expressing shRNA was obtained from Addgene (Cambridge, MA, USA),⁴⁸ and the two shRNA sequences that target 5'-

GCCCCAGTTGAGATGCCTAT-3' (#1577) and 5'-CCCATCCTCTTCCCTATTAAG-3' (#1832) for PRMT5 knockdown and that target 5'-CCACTCCTCAGCCCTTATTA-3' (#2310) for Sp1 knockdown were selected for constructing pLKO-Tet-On-shPRMT5 and pLKO-Tet-On-shSp1 as described previously.³⁰ The pLKO-Tet-On-SC and pFLAG-CMV-AR were constructed before.³⁰ The AR promoter luciferase reporter gene construct and the PSA promoter luciferase reporter gene construct were kindly provided by Dr Donald Tindall. pFLAG-CMV-AR was made by subcloning the AR cDNA into pFLAG-CMV vector. All plasmids were confirmed by DNA sequencing.

Cell proliferation assay

The cell proliferation assay was performed using MTT reagent (Sigma, St Louis, MO, USA). For transient transfection experiments, LNCaP, DU145 or PC-3 cells (4×10^3) were seeded in 48-well plates for 24 h, and then transiently transfected with pLKO-Tet-On-shPRMT5 (#1577) or the SC control using FuGENE HD or FuGENE 6 (Promega, Madison, WI, USA) for 96 h after the transfection. For MTT analysis, cell medium was removed and 70 μl of MTT solution (0.5 mg/ml) was added into each well and incubated at 37 °C for 4 h. At the end of incubation, MTT solution was removed and 130 μl of DMSO was added into each well and incubated at 37 °C for another 10 min. The plates were then read at 560 nm with TECAN Microplate Reader (TECAN, Mannedorf, Switzerland). For LNCaP, DU145, C4-2 and RWPE-1 stable cell lines, similar procedure was followed except that Dox was added at 1 μg/ml to induce PRMT5 knockdown during culture. At least three independent experiments were performed and the mean ± s.d. was presented. Student's *t*-test was performed to determine the statistical significance. The effect of PRMT5 inhibitor BLL3.3 on the growth of LNCaP, DU145 and RWPE-1 cells was similarly determined by MTT.

Soft-agar growth assay

The soft-agar growth assay to measure anchorage-independent proliferation of LNCaP cells was performed by using the 96-well plate format as described previously.⁴⁹ Briefly, LNCaP cells were transfected with pLKO-Tet-On-shPRMT5 (#1577) or pLKO-Tet-On-SC for 24 h, and then 2.5×10^3 cells were added into the middle layer agar. Dox was added into each layer

of soft agar at 1 µg/ml to induce the expression of shRNAs. The plates were incubated at 37 °C, 5% CO₂ for 7 days. To quantify the colony-formation efficiency, 16 µl of AlamerBlue Cell Viability Reagent (Invitrogen, Carlsbad, CA, USA) was added into each well and incubated at 37 °C for another 4 h. Fluorescence intensity was measured at 570EX nm/600EM nm using Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). Experiments were performed in triplicate, and results from three independent experiments were analyzed and presented as mean ± s.d. Student's *t*-test was used to determine the statistical significance.

qRT-PCR and western blotting

To determine the effect of PRMT5 knockdown on AR expression, PRMT5 were transiently or stably knocked down in LNCaP cells for 96 h, and total RNA was isolated using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). One microgram of total RNA was used for reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Promega) according to manufacturer's instruction. The qRT-PCR analysis of AR or AR target genes (PSA, *KLK2*, *TMRSS2*) was performed as described previously.³⁰ Antibodies against AR (SC-816, Santa Cruz, CA, USA), PRMT5 (07-405, Millipore, Billerica, MA, USA), PSA (1984-1, Epitomics, Burlingame, CA, USA), FLAG (Sigma, F-1804), Sp1 (ab13370, Abcam, Cambridge, MA, USA), H4R3me2s (Abcam, ab5823), H3R8me2s (Abcam, ab130740), H2AR3me2s (Abcam, ab22397), and Brg1 (Abcam, ab110641) were used for western blotting analysis.

Chromatin immunoprecipitation assay

The LNCaP stable cell line or parental cells were cultured in the presence or absence of Dox (1 µg/ml) for 96 h. At the end of induction, 270 µl of 37% formaldehyde was added into each dish and incubated at room temperature for 10 min. Then 1 ml of 1.25 M glycine was added to stop the cross-linking reaction. Cells were then harvested, resuspended in 1 ml of immunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 0.5% NP-40, 0.5 mM DTT, and protease and phosphatase inhibitors), and finally sonicated (Branson Sonifier250set, Wilmington, NC, USA) to prepare sheared chromatin. Antibodies against PRMT5 (Millipore, 07-405), Sp1 (Santa Cruz, SC7824), Brg1 (Abcam, ab110641), H4R3me2s (Abcam, ab5823), H3R8me2s (Abcam, ab130740), H2AR3me2s (Abcam, ab22397) and IgG (Santa Cruz, SC2027) were used to immunoprecipitate protein-DNA complexes for isolation of PCR-ready DNA using the Fast ChIP protocol described previously.⁵⁰ The co-immunoprecipitated proximal promoter region of AR (−493 to −226) was quantified by qRT-PCR. Results were normalized to the IgG control and are presented as mean ± s.d. from three independent experiments. Student's *t*-test was used to determine the statistical significance.

Co-immunoprecipitation of PRMT5 with Sp1 and Brg1

Total cell lysate of LNCaP cells was prepared in immunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 0.5% NP-40, 0.5 mM DTT, and protease and phosphatase inhibitors) for co-immunoprecipitation. Anti-PRMT5 antibody or IgG was used to immunoprecipitate PRMT5 from 500 µg of total lysate, and co-immunoprecipitated Sp1 and Brg1 was detected with Sp1 and Brg1 antibodies.

Expression of PRMT5 and AR and the analysis of their correlation in prostate cancer tissues

A TMA consisting of 32 BPH tissues and 40 prostate cancer tissues (20 with Gleason score 6 and 20 with Gleason score ≥ 7) was used for immunohistochemistry analysis of PRMT5 and AR expression. Briefly, paraffin section of the TMA was deparaffinized in xylene and rehydrated in graded ethanol, followed by inactivation of endogenous peroxidase activity in 3% hydrogen peroxide for 10 min. Antigen retrieval was performed by heating slides in 10 mM Tris-HCl (pH 10) for 30 min in microwave. After three washes with phosphate-buffered saline containing 0.1% Tween 20 (PBST), slides were blocked in 5% non-fat milk in PBST at room temperature for 1 h. The primary antibodies against PRMT5 or AR was incubated at 4 °C overnight, followed by three washes with PBST and incubation with HRP-conjugated anti-rabbit secondary antibodies (Amersham, Pittsburgh, PA, USA) at room temperature for 1 h. The signal was developed with diaminobenzidine for 10 min, and sections were counterstained with hematoxyline. The semi-quantification of PRMT5 and AR expression was performed as described previously with slight modifications.⁵¹ The intensity was scored as 0 (no expression), 1 (low expression), 2 (moderate expression) and 3 (high expression), and the percentage of cells showing the expression was scored ranging from 0 to 10

with 10 as the highest percentage (100%). The expression score for cytoplasmic- and nuclear-localized PRMT5 was respectively determined by the intensity score times the percentage (0–30), and the total expression score is the sum of the cytoplasmic and nuclear expression scores (0–60). The unpaired *t*-test was used to determine the statistical significance of the total mean expression score between BPH and prostate cancer tissues, and paired *t*-test was used to determine the difference in expression scores between cytoplasmic-localized PRMT5 and nuclear-localized PRMT5. The same semi-quantification method was used for AR expression in the nucleus.

To determine the correlation between the expression of PRMT5 and AR in the nucleus in prostate tissues, their nuclear expression scores were used for Pearson's analysis. To determine the correlation of PRMT5 and AR expression at the transcript level, we retrieved their expression data from 8 studies that have >60 tissues from Oncomine. The statistic Q was calculated to test the homogeneity of effect sizes across studies for each of the three methods (Pearson's, Spearman's and Kendall's),⁵² and it was found that the effect sizes across studies were not homogeneous (all with *P*-value < 1e − 12). Therefore, we used a random-effects model for the meta-analysis of each method.⁵³

Xenograft tumor growth in nude mice

Animal experiments were approved by the Purdue University Animal Care and Use Committee. Male athymic nude mice (5–7 week old) were purchased from Harlan Laboratories (Indianapolis, IN, USA), and 3 × 10⁶ cells of established stable cell lines that inducibly express PRMT5 shRNA or SC were co-injected subcutaneously into the right lower flank of 20 mice with Matrigel (1:1 in volume). Assuming that PRMT5 knockdown can reduce tumor volume by 30% and that standard deviation within each group is about 25% of the mean tumor volume, a sample size of 10 male mice per group will have over 80% power to detect a 30% difference between the two groups at alpha level 0.05. Mice were randomly divided into two groups (10 mice/group) for each stable cell line by using Excel-based randomization method, and treated with Dox (1 mg/ml in drinking water) or without Dox (drinking water only). Tumor growth was monitored twice weekly, and tumor volume was calculated using $\frac{1}{2} \times L \times W \times H$ without using blinding method. At the end of experiments, tumors were resected and formalin fixed, and paraffin embedded. Immunohistochemistry analysis of PRMT5 and AR expression was similarly performed as described above. We used the following linear mixed model to model the *j*-th observed xenograft tumor volume of *i*-th mouse, that is, y_{ij} , assuming cubic polynomial growth of tumors over time,

$$y_{ij} = \gamma_{i0} + \gamma_{i1} t_j + \gamma_{i2} t_j^2 + \gamma_{i3} t_j^3 + \varepsilon_{ij}, \quad \varepsilon_{ij} \sim N(0, \sigma^2), \quad \gamma_{ik} \sim N(\beta_k + \delta_k D_i, \sigma_k^2),$$

Where, t_j is the number of days after implantation for the *j*-th observation, D_i indicates whether the *i*-th subject is under Dox. The random-effects are independent, and the errors of the same subject are assumed to follow a first-order continuous autoregressive model.

To evaluate the effects of Dox on the tumor growth, we are subject to test the $H_0: \delta_0 = \delta_1 = \delta_2 = \delta_3 = 0$ against H_a : at least one of $\delta_0, \delta_1, \delta_2, \delta_3$ is not zero.

We used the likelihood ratio test (χ^2 -test) to conduct the hypothesis tests.

For PRMT5 knockdown, the *P*-value is 1.9305×10^{-6} . For SC, the *P*-value is 0.1670. Error bar, s.e.m.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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Legends to Supplementary Figures

Figure S1. Transient knockdown of PRMT5 inhibits prostate cancer cell growth in LNCaP cells but not DU145 and PC-3 cells. (a) Knockdown of PRMT5 in the indicated prostate cancer cells transiently transfected with scrambled control (SC) or pLKO-Tet-On-shPRMT5(#1577). (b-d) Transient knockdown of PRMT5 inhibited cell proliferation in LNCaP, but not in DU145 and PC-3 cells assayed by MTT. (e) Transient knockdown of PRMT5 by pLKO-Tet-On-shPRMT5(#1577) significantly inhibited colony formation in soft agar assay when compared with SC.

Figure S2. Western blotting analysis of PRMT5 and AR expression in LNCaP and C4-2 cells. Higher expression level of PRMT5 and AR was observed in C4-2 cells.

Figure S3. Rescue of PRMT5 knockdown-induced growth inhibition by AR overexpression in LNCaP cells. (a) LNCaP-shRNA stable cell line were transfected with pFLAG (Vector) or pFLAG-AR (AR) and cultured for 7 days in the presence of doxycycline (Dox+) or absence of doxycycline (Dox-). Cell growth was determined by Trypan blue staining, and the fold increase of cell growth was determined by dividing the total number of cells at day 7 by the number of cells seeded initially. (b) Cell lysate from (a) was used to determine the total expression of AR using an anti-AR antibody and the expression of PRMT5 using an anti-PRMT5 antibody. (c-e) Similar experiments were performed as described in (a), and total RNA was isolated for qPCR quantification of *PSA*, *TMPRSS2* and *KLK2*. Three independent experiments were performed and mean+SD was presented. The Student's *t*-test was used for *P* value calculation between the indicated two groups.

Figure S4. Inhibition of PRMT5 by a small molecule inhibitor attenuates cell proliferation and reduces AR expression in LNCaP cells. (a) LNCaP cells were treated with 10 μ M of BLL3.3, a selective small molecule inhibitor of PRMT5, and cell growth was determined by MTT. (b) LNCaP cells were incubated with BLL3.3 (10 μ M) for 6 days, and the down-regulation of AR expression and the inhibition of symmetric dimethylation of H4R3 (H4R3me2) by the inhibitor were confirmed by Western blotting. Note that BLL3.3 had no effect on the expression level of PRMT5. (c and d) Similar cell growth experiments were performed for DU145 and RWPE-1 as LNCaP and no inhibitory effect was observed.

Figure S5. Immunoprecipitation of histones H4R3, H3R8 and H2A by methylation-specific antibodies. LNCaP cells were crosslinked and chromatins were fragmented as did for ChIP analysis except that proteins were not digested with protease K. Antibodies that recognize H4R3me2s, H3R8me2s and H2AR3me2s were used to immunoprecipitate H4R3, H3R8 and H2A, respectively. All three histones were efficiently immunoprecipitated when compared with the IgG control.

Figure S6. Effect of PRMT5 knockdown on the methylation status of histones. The established doxycycline (Dox)-inducible PRMT5 knockdown cell line LNCaP-shPRMT5 was induced by Dox (1 μ g/ml) for 96 h (Dox+) or without Dox induction (Dox-), and total cell lysate was prepared for Western blotting analysis of H4R3me2s, H3R8me2s, and H2AR3me2s.

Figure S7. Expression of PRMT5 in the cytoplasm and nucleus in prostate tissues. The expression score of both cytoplasmic and nuclear expression of PRMT5 in a prostate cancer TMA was semi-quantified, and the paired *t*-test was used to determine the statistical significance in the subcellular localization of PRMT5 in both BPH (32 cases), prostate cancer tissues with Gleason score 6 (20 cases), and prostate cancer tissues with Gleason score 7 and above (20 cases).

Figure S1

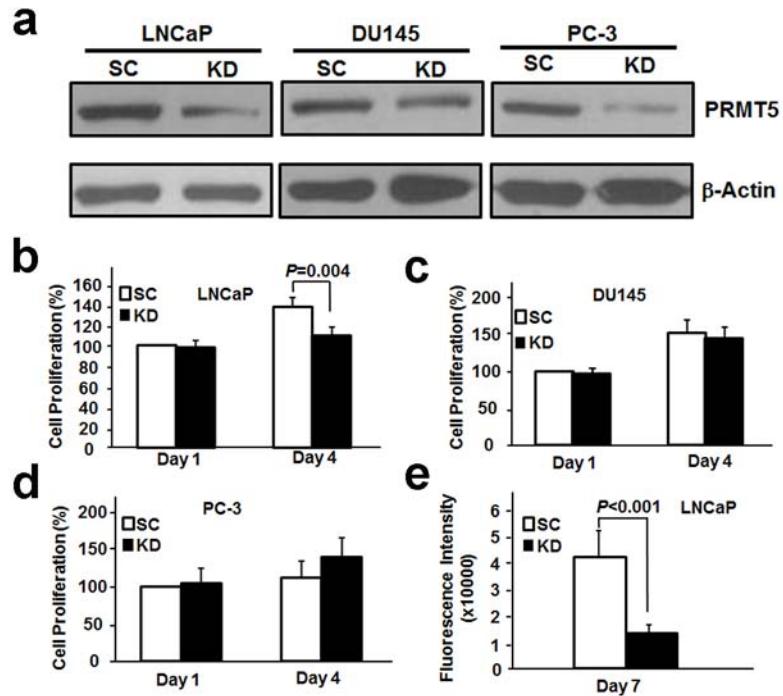


Figure S2

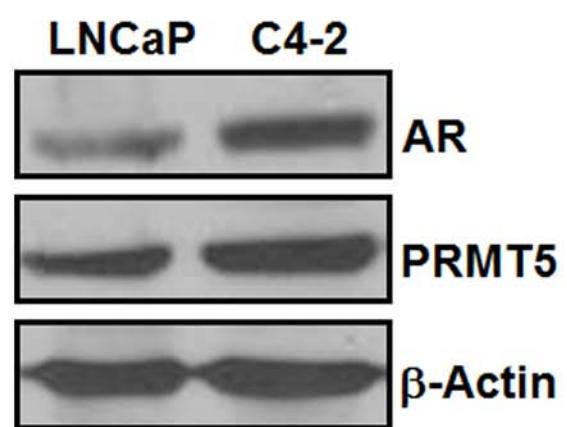


Figure S3

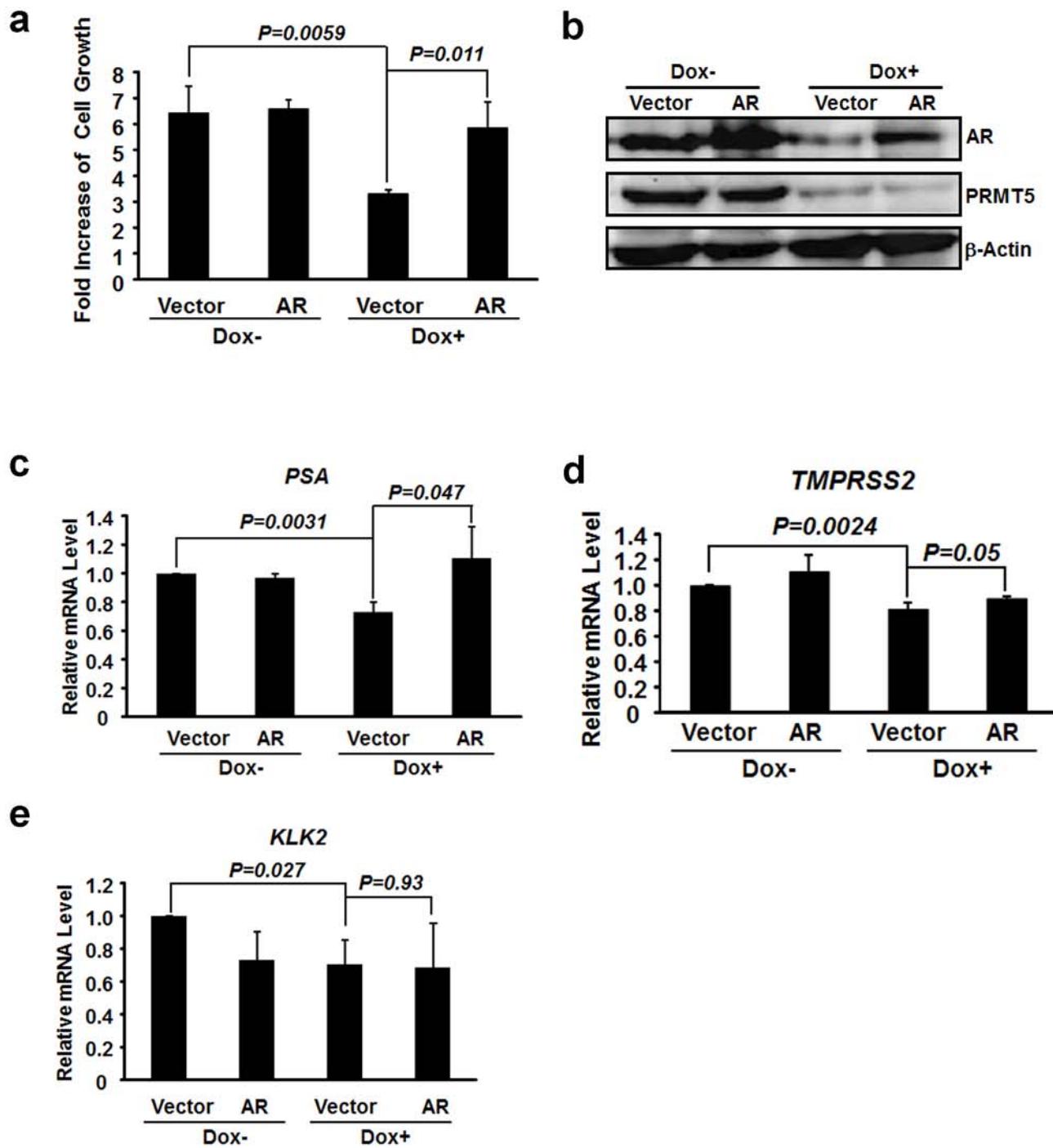


Figure S4

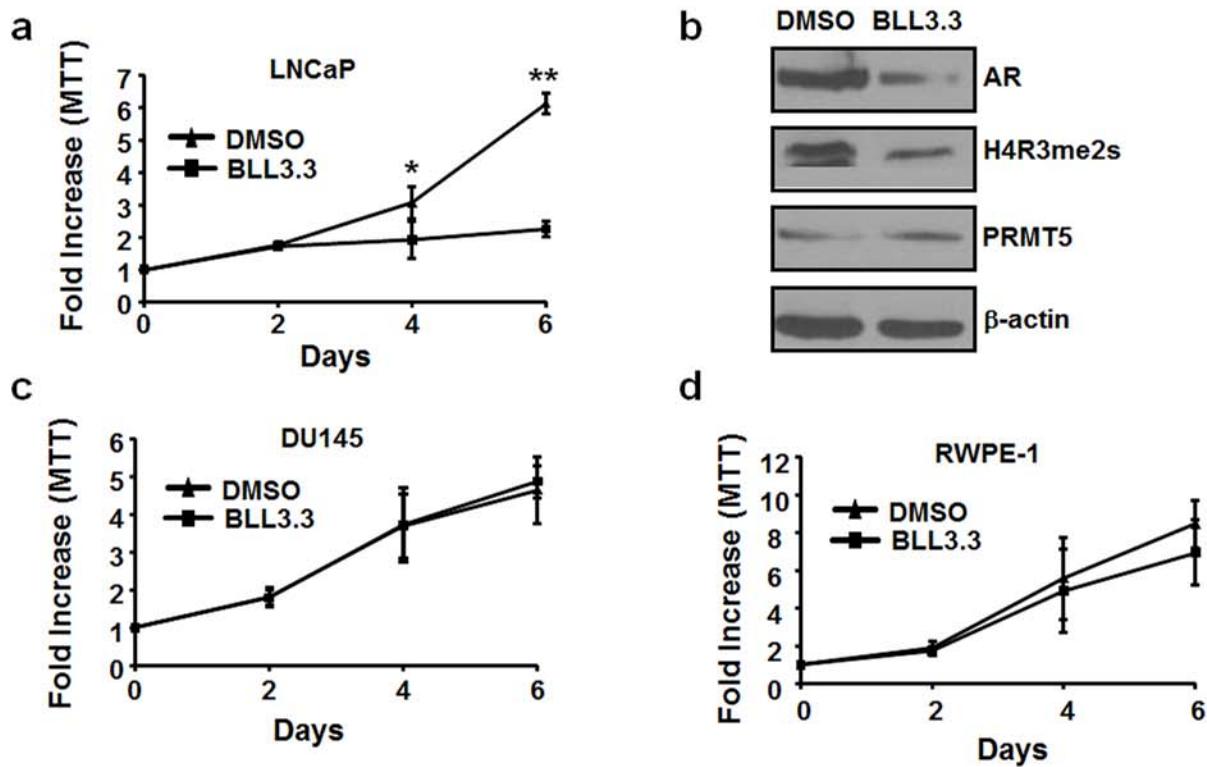


Figure S5

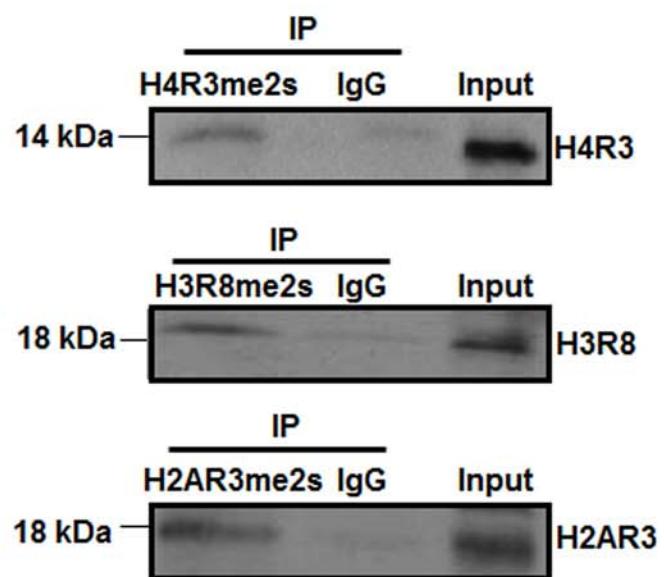


Figure S6

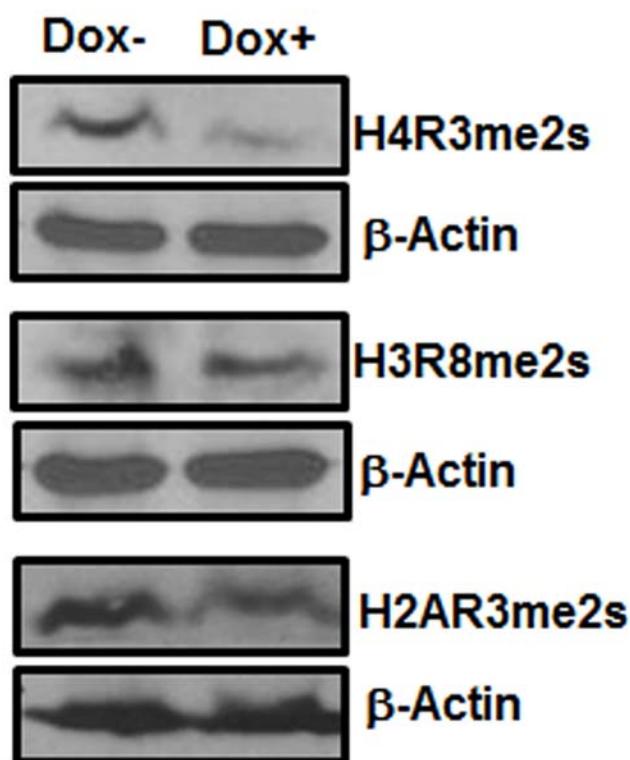
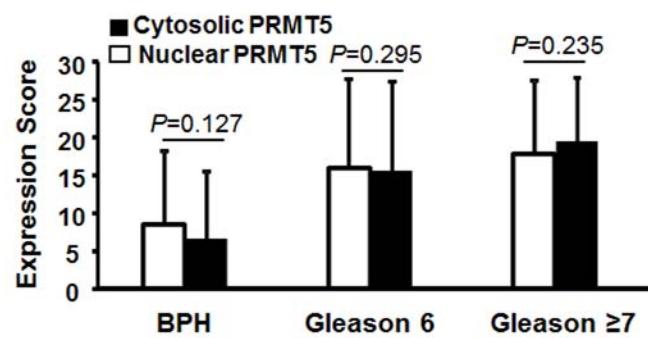


Figure S7



Curriculum Vitae

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(2). Epidemiological investigation of drinking water and cancer incidence in Wuhan, China.

9/1991-3/1994: **Visiting Research Associate** in the Department of Molecular Oncology, Kyoto University School of Medicine, Kyoto, Japan.
(1). Spontaneous and induced acquisition of tumorigenicity in nude mice by lymphoblastoid cell line derived from patients with xeroderma pigmentosum group A.
(2). Subtractive isolation of genes contributing to the acquisition of tumorigenicity by lymphoblastoid cell line derived from xeroderma pigmentosum group A patient.

4/1994-3/1997: **Graduate Student (Ph.D.)** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan
(1). Identification of cysteine-rich domain in Raf-1 as a novel Ras binding domain for activation by Ha-Ras and Rap1A.

- (2). Activation mechanisms of Ras effectors (Raf-1, B-Raf, adenylyl cyclase).

4/1997-8/2000: **Assistant Professor** in the Department of Physiology II, Kobe University School of Medicine, Kobe, Japan.

- (1). Differential regulation of Raf kinase activity by Ha-Ras and Rap1A.
- (2). Identification and characterization of novel Ras effectors, (RalGDS, AF-6, PLC- ϵ) and regulators (RA-GEF-1, RA-GEF-2).
- (3). Activation mechanisms of Ras effectors.

9/2000-6/2003: **Research Investigator/Specialist** in the Department of Biological Chemistry and Howard Hughes Medical Institute, University of Michigan School of Medicine.

- (1). Development of bimolecular fluorescence complementation (BiFC) and multicolor BiFC assays for visualization of protein-protein interactions in living cells.
- (2). Functional analysis of cross-family transcription factor interactions among bZIP, Rel, Smad and Myc/Max families.

7/2003-6/2009: **Assistant Professor** in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

- (1) Development and improvement of BiFC-based technologies
- (2) BiFC analysis of AP-1 dimers in living cells and *C. elegans*
- (3) AP-1 in prostate cancer development and therapeutic responses

7/2009- 7/2015: **Associate Professor** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

- (1) Development and improvement of BiFC-based technologies
- (2) AP-1 in prostate cancer development and progression
- (3) Mechanisms and targeting of radiation-induced neuroendocrine differentiation in prostate cancer
- (4) Protein arginine methyltransferase 5 (PRMT5) in prostate cancer development, progression and therapeutic response

8/2015- present: **Professor** (tenured) in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy.

- (1) Mechanisms and targeting of radiation-induced neuroendocrine differentiation (NED) in prostate cancer
- (2) Role and targeting of protein arginine methyltransferase 5 (PRMT5) in castration resistant prostate cancer (CRPC) and neuroendocrine prostate cancer (NEPC)
- (3) Development of high throughput screens for small molecule inhibitors targeting protein-protein interactions
- (4) Development of BiFC-based cDNA library screens for interacting proteins

08/2013-present: Program Co-Leader of the Cell Identity and Signaling (CIS) program of the Purdue University Center for Cancer Research (PCCR)

08/2013-present: Executive Committee Member of PCCR

08/2010-present: Co-Leader of the Prostate Cancer Discovery Group of PCCR
2011-present: Director of Pharmacy Live Cell Imaging Facility (PLCIF)
7/2016-present: Showalter Faculty Scholar of Purdue University

Current Professional Memberships

2001- Present	American Association for Cancer Research
2009- Present	Society for Basic Urological Research
2010- Present	American Urological Association
2015-present	Radiation Research Society

Awards:

09/91-09/92:	Fellowship of JSPS Source: Japan Society for the Promotion of Science (JSPS)
09/92-09/93:	Kyoto University Alumni Fellowship Source: Kyoto University
04/94-03/97	Senshukai Scholarship (Ph.D. student) Source: Kobe Senshukai Scholarship Foundation
04/98-03/99	President Young Investigator Award Source: Kobe University
04/98-03/99	Young Investigator Award Source: JSPS
04/99-03/01	Young Investigator Award Source: Hyogo Prefecture Science and Technology Association
07/03-08/06	Walther Assistant Professor
07/16-06/21	University Showalter Faculty Scholar Award of Purdue University
04/17	Pharmaceutical Sciences Teacher of the Year (College of Pharmacy)

Professional Services:

Reviewer for Grant Applications

2004	Reviewer of MAES (The Maryland Agricultural Experiment Station at the University of Maryland)
2005	Reviewer for NSF Advisory Panel for Molecular and Cell Biology
2006-2008	American Heart Association (MCB Panel)
2007-2011	Qatar National Research Fund (QNRF)
2008-present	Pennsylvania Department of Health (PADOH)
2008	UK Cancer Research
2008	UK Diabetes
2009	Welcome Trust

2010-2014	Department of Defense, Prostate Cancer Research Program (Immunology, Endocrine, Experimental Therapeutics panels)
2015/2016	Florida Department of Health
2015	NIH, RTB study section (IAR)
2016	NCI (DP5)

Reviewer for Professional Journals

Combinatory Chemistry and HTS, Zebrafish, Journal of Biological Chemistry, Molecular and Cellular Biology, Nature Biotechnology, Nature Methods, Molecular Cell, Molecular Biology of the Cell, PNAS, BMC Biotechnology, BMC Biology, Biotechniques, Biochemistry, ACS Chemical Biology, Chemistry & Biology, Journal of Innovative Optical Health Sciences, TIBS, TIBT, Current Cancer Drug Targets, Journal of Cell Science, PLoS One, Ontarget, Oncogene, Redox Biology, Cancer Letters

Editorial Board Member:

- 2007- Perspective in Medicinal Chemistry
- 2011- American Journal of Cancer Research
- 2013- Journal of Biological Methods (Founding Editorial Member)
- 2014- Frontier in Surgical Oncology (review editor)
- 2015- Journal of Drug Research and Development

Organizer/Program Committee Member/Session Chair of Conferences, Symposia, and Workshops

- Organizer of Tristate Worm Meeting at Purdue (2006)
- Session Chair of Optical Molecular Imaging of the 2008 PIBM
- Session Chair of Imaging Technology Symposium of the 2008 4th Modern Drug Discovery and Development Summit
- Program Member of the 2009 PIBM Program Committee
- Organizer of 2010 Bimolecular Fluorescence Complementation Workshop (Purdue University)
- Member of the Scientific Program Committee and Moderator of Breakout Panel Discussion of the 2013 Drug Discovery Chemistry-Sixth Annual Protein-Protein Interactions, San Diego
- Organizer, Program Committee Member and Session Chair of the 2013 Hefei Prostate Cancer Translational Medicine and Personalized Medicine Symposium
- Session Co-chair of the 2016 Spring SBUR Symposium

Member of Big Ten Cancer Research Consortium (BTRC) GU Clinical Trial Working Group (2013-present)

Consultation on BiFC technology

Since 2003, we have been providing BiFC plasmids, letters of support and consultations to many BiFC users worldwide. The lab provided BiFC plasmids to more than 200 labs prior to 2007. To facilitate the request process, we deposited 11 BiFC plasmids to Addgene in 2007, and more than 2000 requests have been completed via Addgene.

Invited Seminars/Presentations

07/04/17	Place: China Jiliang University School of Pharmacy Title: Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery
06/16/17	Place: Hong Kong University School of Chinese Medicine Title: Bimolecular fluorescence complementation (BiFC): From basic research to drug discovery
06/12/17	Place: Jinan University School of Medicine Title: Protein arginine methyltransferase 5 (PRMT5): An emerging oncogene and therapeutic target in prostate cancer
05/15/2017	Place: Northwestern University School of Medicine, Department of Pathology Title: Neuroendocrine differentiation of prostate cancer: An emerging mechanism of therapy resistance
10/11/2016	Place: Chromatin and Epigenetics Symposium (Purdue) Title: PRMT5 is a master epigenetic activator of DNA damage response and a therapeutic target for prostate cancer radiosensitization (presented by Jake Owens)
05/10/16	Place: 2016 American Urological Association (AUA) meeting Title: Protein arginine methyltransferase 5 (PRMT5) is a novel epigenetic regulator of androgen receptor in prostate cancer
01/07/16:	Place: Jinan University the first affiliated hospital Title: How to conduct scientific research
12/27/15:	Place: Northwest University of Agriculture and Forestry Title: Bimolecular fluorescence complementation (BiFC): Current status and future perspectives
01/05/15:	Place: Tongling First People's Hospital Title: Advances in prostate cancer diagnosis and treatment- A comparative analysis between China and America
12/29/14	Place: Jinan University the first affiliated hospital Title: Targeting PRMT5 for prostate cancer radiosensitization
05/18/14	Place: Mayo Clinic, Departments of Radiation Oncology Title: Mechanism and targeting of radiotherapy-induced neuroendocrine differentiation for prostate cancer treatment
03/25/14	Place: Tongling 4 th Hospital, Wannan Medical College Title: Advances in prostate cancer diagnosis and treatment
02/27/14	Place: UCLA, Departments of Pathology and Laboratory Medicine

10/9/13	<p>Title: Targeting neuroendocrine differentiation as a novel radiosensitization approach for prostate cancer treatment</p> <p>Place: Cancer Hospital, Hefei Institutes of Physical Science Chinese Academy of Sciences</p>
05/24/13	<p>Title: Development of radiosensitizers: An urgent need for prostate cancer radiotherapy</p> <p>Place: Hefei Chinese Academy of Sciences Cancer Hospital</p>
05/20/13	<p>Title: Impact of neuroendocrine differentiation in prostate cancer radiotherapy</p> <p>Place: Huazhong University of Science and Technology Union Hospital Cancer Institute</p>
05/17/13	<p>Title: Radiation-induced neuroendocrine differentiation in prostate cancer: From bench to bedside</p> <p>Place: Jinan University School of Medicine</p>
05/14/13	<p>Title: Neuroendocrine differentiation (NED) in prostate cancer cells: From basic science to clinical practice</p> <p>Place: Northwestern Agriculture and Forestry University (NWAFU): 2013 Purdue-NWAFU Center Symposium</p>
04/17/13	<p>Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives</p> <p>Place: 2013 Drug Discovery Chemistry in San Diego: Sixth Annual Protein-Protein Interactions (Targeting PPI for Therapeutic Interventions)</p> <p>Title: Bimolecular fluorescence complementation (BiFC) as a novel imaging-based screening for inhibitors of protein-protein interactions.</p>
02/05/13	<p>Place: Tongji Hospital, Huazhong University of Science and Technology</p> <p>Title: Neuroendocrine differentiation (NED): A therapeutic challenge in prostate cancer management</p>
10/25/12	<p>Place: Wright State University Department of Biochemistry and Molecular Biology</p> <p>Title: Bimolecular fluorescence complementation (BiFC): An imaging tool for visualization of molecular events</p>
06/06/12	<p>Place: Jiangshu University School of Medical Technology and Laboratory Medicine</p> <p>Title 1: Mechanisms and targeting of radiation-induced neuroendocrine differentiation</p> <p>Title 2: Bimolecular fluorescence complementation (BiFC): Past, Present and Future</p>
06/4/12	<p>Place: Chinese Academy of Sciences (Hefei)</p> <p>Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future</p>
05/31/12	<p>Place: Tongling Traditional Chinese Medicine Hospital</p> <p>Title: Recent advances in prostate cancer diagnosis and treatment</p>

05/18/12	Place: Shanghai Center for Plant Stress Biology of Chinese Academy of Sciences Title: Bimolecular fluorescence complementation (BiFC): Past, Present and Future
04/25/12	Place: University of Western Ontario Title: Radiotherapy-induced neuroendocrine differentiation: Implications in prostate cancer progression and treatment
03/13/12	Place: Mayo Clinic Department of Urology Title: Mechanisms and targeting of therapy-induced neuroendocrine differentiation for prostate cancer treatment
07/11/11	Place: Jinan University Medical School Title: Bimolecular fluorescence complementation: An emerging technology for biological research
07/10/11	Place: Sun-Yat-sun University Medical School Title: Mechanisms and targeting of therapy-resistant prostate cancer
02/09/11	Place: Tulane University Medical School Title: Mechanisms and targeting of therapy-resistant prostate cancer
01/17/11	Place: Penn State University College of Medicine Title: Bimolecular fluorescence complementation (BiFC): Current Challenges and Future Developments
12/07/10	Place: Purdue University BiFC Workshop Title: Bimolecular fluorescence complementation: principle, experimental design and data analysis
11/18/10	Place: UT Austin College of Pharmacy Title: Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimerization in living cells and <i>C. elegans</i>
09/28/10	Place: Nanjing University Medical School Title: Multicolor bimolecular fluorescence complementation (BiFC): A novel high throughput screening method for protein-protein interactions
09/25/10	Place: Wannan Medical College Title: Mechanisms and targeting of therapy-resistant prostate cancer
09/16/10	Place: Wuhan Institute of Virology Title: Bimolecular fluorescence complementation (BiFC): Current Status and Future Perspectives
09/13/10	Place: Beijing University Cancer Hospital Title: Mechanisms and targeting of therapy resistant prostate cancer
09/08/10	Place: Purdue University BIG Symposium Title: Fluorescence complementation: An emerging tool for visualization of molecular events in living cells and animals
10/16/09	Place: Southern China Agriculture University

	Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
10/19/09	Place: Sun Yat-sen University Zhongshan Medical School
	Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
10/26/09	Place: Bengbu Medical College
	Title: Principle and applications of bimolecular fluorescence complementation (BiFC)
10/28/09	Place: Nanjing University Medical School
	Title: Seeing is believing: visualization of protein-protein interactions using bimolecular fluorescence complementation (BiFC),
05/07/09	Place: University of Chicago Graduate Program of Physiology
	Title: Bimolecular fluorescence complementation (BiFC) analysis in living cells and living animals,
02/02/09	Place: Indiana University Medical School, Department of Biochemistry
	Title: Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy
12/08/08	Place: University of Virginia Cancer Center
	Title: Ionizing radiation-induced neuroendocrine differentiation: implication in prostate cancer therapy
11/25/08	Place: 7 th International Conference on Photonics and Imaging in Biology and Medicine (Wuhan, China), Nov 24-27, 2008
	Title: Fluorescence complementation: an emerging technology in biomedical research (presentation and panel discussion)
10/15/08	Place: 4 th Modern Drug Discovery & Development Summit (San Diego, 10/15/08-10/17/08)
	Title: Multicolor bimolecular fluorescence complementation in drug discovery
11/29/07	Place: UMDNJ-SOM Stratford
	Title: Bimolecular fluorescence complementation (BiFC) analysis of AP-1 dimerization in living cells and living animals
11/28/07	Place: The Children's Hospital of Philadelphia and the University of Pennsylvania
	Title: Molecular regulation and targeting of ATF2 nucleocytoplasmic shuttling
11/13/07	Place: Department of Biochemistry, Purdue University
	Title: AP-1 biology, pathology, and technology
10/30/07	Place: Fluorescent proteins and Biosensors Symposium at HHMI Janelia Farm
	Title: BiFC-FRET, a novel assay for visualization of ternary complexes in living cells
08/07/07	Place: International Microscopy & Microanalysis 2007 at Ft. Lauderdale
	Title: Bimolecular fluorescence complementation (BiFC) and

		beyond
02/09/07	Place:	Montana State University Department of Microbiology
	Title:	Functional analysis of AP-1 dimerization by bimolecular fluorescence complementation
11/01/06	Place:	Vanderbilt University Institute of Chemical Biology
	Title:	Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system
10/04/06	Place:	University of Illinois at Chicago School of Medicine
	Title:	Bimolecular fluorescence complementation: principle and applications
07/17/06	Place:	Huazhong University of Science and Technology Tongji Medical College
	Title:	Bimolecular fluorescence complementation: principle and applications
03/14/06	Place:	University of Toronto Western Research Institute
	Title:	Visualization of AP-1 protein interactions in living cells and in living animals using an improved BiFC system
09/30/05	Place:	Eli Lilly, Indianapolis
	Title:	Identification of new fluorescent protein fragments for BiFC analysis under physiological conditions
03/10/05	Place:	Purdue University, School of Health Science, Purdue University
	Title:	Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
09/02/04	Place:	Illinois State University, Department of Biology
	Title:	Role of <i>C. elegans</i> Fos and Jun homologs in development.
08/13/04	Place:	Cold Spring Harbor (Cold Spring Harbor Image Course)
	Title:	Seeing is believing: visualization of transcription factor interactions in living cells and in living animals using a novel using bimolecular fluorescence complementation (BiFC) approach
05/07/04	Place:	Purdue University, Department of Chemistry
	Title:	Seeing is believing: visualization of transcription factor interactions in living cells and in living animals
01/14/04	Place:	Purdue University, Department of Biological Science
	Title:	Seeing is believing: visualization of transcription factor interactions in living cells and in living animals
12/04/03	Place:	Indiana University at Bloomington, Department of Biology
	Title:	Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
11/07/03	Place:	Purdue Cancer Center (Purdue Cancer Center Director's Advisory council)
	Title:	Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions in cancer research
09/04/03	Place:	Purdue Cancer Center (Annual Scientific Retreat)

	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interactions
03/11/03	Place: Cincinnati Children's Hospital, Division of Experimental Hematology
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
03/04/03	Place: Harvard Medical School, MGH, Laboratories of Photomedicine
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
02/24/03	Place: Medical University of South Carolina, School of Pharmacy Department of Pharmaceutical Science
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
02/19/03	Place: University of Texas M.D. Anderson Cancer Center, Department of Molecular Therapeutics
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
02/06/03	Place: Ohio State University, School of Medicine Department of Physiology and Cell biology
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
12/28/02	Place: Purdue University Cancer Center
	Title: Bimolecular fluorescence complementation (BiFC), a novel approach to study protein-protein interaction in living cells
07/20/00	Place: Bengbu Medical College, Bengbu, China
	Title: Recent progress in the activation mechanisms of Raf by Ras
07/15/00	Place: Tongji Medical University, Wuhan, China
	Title: Cloning and functional characterization of a novel type phospholipase C (PLC-ε)

Development of Intellectual Property

- A novel fluorescent protein for protein-protein interaction studies, 65557.P1.US Patent filed on July 16, 2010
- Methods for identifying protein-protein interactions, 66261-01-2013 US Patent filed on June 13, 2013
- Methods for identifying protein-protein interactions, 66261-02-2014 US Patent filed on June 14, 2014
- Bimolecular fluorescence complementation (BiFC)-based screen for discovery of PRMT5 inhibitors. Provisional Patent Application No 62/121,627 filed on February 27, 2015

Publications

a. Peer-reviewed Research Articles

Deng, X., Shao, G., Zhang, H.T., Li, C., Zhang, D., Cheng, L., Elzey, B.D., Pili, R., Ratliff, T.L., Huang, J., Hu, C.D. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. *Oncogene*, 36:1223-1231 (2017)

Vickman, R.E., Christ, S.A., Kerian, K., Eberlin, L., Coos, R.G., Burcham, G.N., Buhman, K.K., Hu, C.D., Mesecar, A.D., Cheng, L., Ratliff, T.L. Cholesterol sulfonation enzyme, SULT2B1b, modulates AR and cell growth properties in prostate cancer. *Mol Cancer Res*, 14:776-786 (2016)

Zhang, H., Zeng, L., Tao, A.W., Zha, Z., and Hu, C.D*. The E3 ubiquitin ligase CHIP mediates ubiquitination and proteasomal degradation of PRMT5. *Biochem Biophys Acta*, 1863:336-346 (2016)

Xu, D., Zhan, Y., Qi, Y., Cao, B., Bai, S., Xu, W., Gambhir, S.S., Lee, P., Sartor, O., Flemington, E.K., Zhang, H., Hu, C.D., and Dong, Y. Androgen receptor splice variants dimerize to transactivate target genes. *Cancer Res*, 75:3663-3671 (2015)

Suarez, C.D., Deng, X., and Hu, C.D.* Targeting CREB inhibits radiation-induced neuroendocrine differentiation and increases radiation-induced cell death in prostate cancer cells. *Am J Cancer Res*, 4:850-861 (2014)

Zhang, H., Zha, Z. and Hu, C.D*. Transcriptional activation of PRMT5 by NF-Y is required for cell growth and negatively regulated by the PKC/c-Fos signaling in prostate cancer cells. *Biochem Biophys Acta*, 1839:1330-1340 (2014)

Hsu, C. and Hu, C.D.* Transcriptional activity of c-Jun is critical for the suppression of AR function. *Mol. Cell. Endocrinol.* 372:12-22 (2013)

Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. *J. Biol. Chem.* 287:12455-12688 (2012)

Hsu, C. and Hu, C.D.* Critical role of an N-terminal end nuclear export signal in regulation of ATF2 subcellular localization and transcriptional activity. *J. Biol. Chem.* 287:8621-8632 (2012)

Deng, X., Elzey, B.D., Poulson, J.M., Morrison, W.B., Ko, S.C., Hahn, N.M., Ratliff, T.L., and Hu, C.D.* Ionizing radiation induces neuroendocrine differentiation in vitro, in vivo and in human prostate cancer patients. *Am. J. Cancer. Res.* 1:834:844 (2011)

Xing, J., Wang, S., Lin, F., Pan, W., Hu, C.D., and Zheng, C. A comprehensive characterization of interaction complexes of Herpes Simplex Virus type 1 ICP22, UL3, UL4 and UL20.5. *J. Virol.* 85:1881-1886 (2011)

Kodama, Y. and Hu, C.D.* An improved bimolecular fluorescence complementation assay with high signal-to-noise ratio. *Biotechniques*, 49:793-805 (2010)

Le, T.T, Duren, H.M., Slipchenko, M.N., Hu, C.D.* and Cheng, J.X. Label-free quantitative analysis of lipid metabolism in living *Caenorhabditis elegans*. *J. Lipid Res.* 51:672-677 (2010)

Hiatt, S.M., Duren, H.M. Shyu, Y., Ellis, R.E., Hisamoto, N., Matsumoto, K., Kariya, K., Kerppola, T.K., and Hu, C.D.* *C. elegans* FOS-1 and JUN-1 regulate *plc-1* expression to control ovulation. *Mol. Biol. Cell* 20:3888-3895 (2009)

Xu, Y., Yang W.H., Gerin, I., Hu, C.D., Hammer, G.D., and Koenig, R.J. DAX-1 and steroid receptor RNA activator (SRA) function as transcriptional coactivators for steroidogenic factor-1 in steroidogenesis. *Mol. Cell. Biol.* 29:1719-1734 (2009)

Yuan, Z., Gong, S., Song, B., Mei, Y., Hu, C., Li, D., Thiel, G., Hu, C.D., and Li, M. Opposing role for ATF2 and c-Fos in c-Jun-mediated apoptosis induced by potassium deprivation in cerebellar granule neurons. *Mol. Cell. Biol.* 29:2431-2442 (2009)

Deng, X., Liu, H., Huang, J., Cheng, L., Keller, E.T., Parsons, S.J., and Hu, C.D.* Ionizing radiation induces prostate cancer neuroendocrine differentiation through interplay of CREB and ATF2: Implications for disease progression. *Cancer Res.* 68:9663-9670 (2008)

Hiatt, S.M., Shyu, Y., Duren, H.M, and Hu, C.D.* Bimolecular fluorescence complementation (BiFC) analysis of protein interactions in living *C. elegans*. *Methods*, 45:185-191 (2008)

Vidi, P.A., Chemel, B.R., Hu, C.D., Watts, V.J. Ligand-Dependant Oligomerization of Dopamine D₂ and Adenosine A_{2A} Receptors in Living Neuronal Cells. *Mol. Pharmacol.* 74:544-551 (2008)

Shyu, Y., Suarez C.D., and Hu, C.D.* Visualizing ternary complexes in living cells using BiFC-FRET analysis. *Nat. Protocol.* 3:1693-1702 (2008).

Shyu, Y., Fox, SM., Duren, HM., Ellis, R.E., Kerppola, T.K. and Hu, C.D.* Visualization of protein interaction in living *Caenorhabditis elegans* using bimolecular fluorescence complementation (BiFC) analysis. *Nat Protocol.*, 4:588-596 (2008).

Shyu, Y., Suarez, C., and Hu, C.D.* Visualization of AP-1-NF-κB ternary complexes in living cells by using a BiFC-based FRET. *Proc Natl Acad Sci U.S.A.*, 105:151-156 (2008).

Tong, E.H.Y., Guo, J.J., Haung, A., Liu, H., Hu, C.D., Chung, S.S.M., and Ko, C.B. Regulation of nucleocytoplasmic trafficking of transcription factor OREBP/TonEBP/NFAT5. *J. Biol. Chem.* 281:23870-23879 (2006).

Wang ,KZQ, Wara-Asparati, N., Boch, J.A., Yoshida, Y., Hu, C.D., Galson, D.L., and Auron, P.E. TRAF6 activation of PI3 kinase-dependent cytoskeletal changes is cooperative with Ras and mediated by an interaction with cytoplasmic c-Src. *J. Cell Sci.* 119:1579-1591 (2006).

Liu, H., Deng, X., Shyu, Y., Li, J.J., Taparowsky, EJ., and Hu, C.D.* Mutual regulation of c-Jun and ATF2 by transcriptional activation and subcellular localization. *EMBO J.*, 25:1058-1069 (2006).

Shyu, Y., Liu, H., Deng, X., and Hu, C.D.* Identification of new fluorescent fragments for BiFC analysis under physiological conditions. *BioTechniques*, 40:61-66 (2006).

Grinberg A., Hu, C.D., and Kerppola T. Visualization of Myc/Max/Mad family dimers and the competition for dimerization in living cells. *Mol. Cell Biol.* 24, 4294-4308 (2004).

Hu, C.D. and Kerppola, T. Simultaneous visualization of interactions between multiple proteins in living cells using multicolor bimolecular fluorescence complementation analysis. *Nat. Biotechnol.* 21, 539-545 (2003).

Hu, C.D., Chinenov, Y., and Kerppola, T Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell.* 9, 789-798 (2002).

Gao X., Satoh T., Liao Y., Song C., Hu, C.D., Kariya K., and Kataoka T. Identification and characterization of RA-GEF-2, a Rap guanine nucleotide exchange factor that serves as a downstream target of M-Ras. *J. Biol. Chem.* 276, 42219-42225 (2001).

Jin T.-G., Satoh T., Liao Y., Song C., Gao X., Kariya K., Hu, C.D., and Kataoka T. Role of the CDC25 homology domain of phospholipase C-epsilon in amplification of Rap1-dependent signaling. *J. Biol. Chem.* 276, 30301-30307 (2001).

Song[#], C., Hu[#], C.D., Masago, M., Kariya, K., Yamawaki-Katayama, Y., Shibatohge, M., Sen, H., Wu, D., Satoh, T., and Kataoka, T. Regulation of a novel human phospholipase C, PLC- ϵ through differential membrane targeting by Ras and Rap1 *J. Biol. Chem.* 276, 2752-2757 (2001). [#]Equal contribution to this work

Liao, Y., Satoh, T., Gao, X., Jin, T.-G., Hu, C.D., and Kataoka, T. RA-GEF-1, a guanine nucleotide exchange factor for Rap1, is activated by translocation induced by association with Rap1GTP and enhances Rap1-dependent B-Raf activation. *J. Biol. Chem.* 276, 28478-28483 (2001).

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regulation of Ras-dependent Raf-1 activation. *Biochem. Biophys. Res. Commun.*, 271, 596-602 (2000).

Shima, F., Okada, T., Kido, M., Sen, H., Tanaka, Y., Tamada, M., Hu, C.D., Yamawaki-Kataoka, Y., Kariya, K., and Kataoka, T. Association with CAP forms a second Ras-binding site of yeast adenylyl cyclase which mediates activation by posttranslationally modified Ras protein. *Mol. Cell. Biol.* 20, 26-33 (2000).

Liao, Y., Kariya, K., Hu, C.D., Shibatohge, M., Goshima, M., Okada, T., Watari, Y., Gao, X., Jin, T.-G., Yamawaki-Kataoka, Y., and Kataoka, T. RA-GEF, a novel Rap1A guanine nucleotide exchange factor containing a Ras/Rap1A-associating domain, is conserved between nematode and humans. *J. Biol. Chem.* 274, 37815-37820 (1999).

Tanaka, Y., Minami, Y., Mine, S., Hirano, H., Hu, C.D., Fujimoto, H., Fujii, K., Saito, K., Tsukada, J., van Kooyk, Y., Figdor, C. G., Kataoka, T., and Eto, S. H-Ras signals to cytoskeletal machinery in induction of integrin-mediated adhesion of T cells. *J. Immunol.*, 163, 6209-6216 (1999).

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Watari, Y., Kariya, K., Shibatohge, M., Liao, Y., Hu, C.D., Goshima, M., Tamada, M., Kikuchi, A., and Kataoka, T. Identification of Ce-AF-6, a novel *Caenorhabditis elegans* protein, as a putative Ras effector, *Gene*, 224, 53-58 (1998).

Shibatohge, M., Kariya, K., Liao, Y., Hu, C.D., Watari, Y., Goshima, M., Shima, F., and Kataoka, T. Identification of PLC210, a *C. elegans* homolog of phospholipase C, as a putative effector of Ras, *J. Biol. Chem.*, 273, 6218-6222 (1998).

Shirouzu, M., Morinaka, K., Koyama, S., Hu, C.D., Hori-Tamura, N., Okada, T., Kariya, K., Kataoka, T., Kikuchi, A., and Yokoyama, S. Interactions of the amino acid residue at position 31 of the c-Ha-Ras with Raf-1 and RalGDS, *J. Biol. Chem.*, 273, 7737-7742 (1998).

Ohnishi, M., Yamawaki-Kataoka, Kariya, K., Tamada, M., Hu, C.D., and Kataoka, T. Selective inhibition of Ras interaction with its particular effector by synthetic peptides corresponding to the Ras effector region, *J. Biol. Chem.*, 273, 10210-10215 (1998).

Yanagihara, C., Shinkai, M., Kariya, K., Yamawaki-Kataoka, Y., Hu, C.D., Masuda, T., and Kataoka, T. Association of elongation factor 1 α and ribosomal protein L3 with the proline-rich region of yeast adenylyl cyclase-associated protein CAP. *Biochem. Biophys. Res. Commun.*, 232, 503-507(1997).

Hu, C.D., Kariya, K., Kotani, G., Shirouzu, M., Yokoyama, S., and Kataoka, T. Coassociation of Rap1A and Ha-Ras with Raf-1 N-terminal region interferes with Ras-dependent activation of Raf-1. *J. Biol. Chem.*, 272, 11702-11705 (1997).

Tamada, M., Hu, C.D., Kariya, K., Okada, T., and Kataoka, T. Membrane recruitment of Raf-1 by association is not only the major function of Ras in Raf-1 activation, *Oncogene*, 15, 2959-2964 (1997).

Hu, C.D., Kariya, K., Tamada, M., Akasaka, K., Shirouzu, M., Yokoyama, S., and Kataoka, T. Cysteine-rich region of Raf-1 interacts with activator domain of post-translationally modified Ha-Ras. *J. Biol. Chem.*, 270, 30274-30277 (1995).

Hu, C.D.*, Zhan, Z.-L., and He, S.-P. Study on the mutagenicity of trichloromethane. *Chinese J. Public Health*, 5, 220-222 (1990) (in Chinese).

Hu, C.D.*, Zhan, Z.-L. and He, S.-P. Study on the influential factors and the sensitivity of microtitre fluctuation test. *Journal of Healthy and Toxicology*, 4, 115-118 (1990) (in Chinese).

Hu, C.D.* and Zhang, X.-H. Influence of EM on spleen cells NK activity and its mechanisms. *Chinese Journal of Microbiology and Immunology*, 8, 11-14 (1989) (in Chinese).

Hu, C.D. * and Zhang, X.-H. Influence of EM on specific immune responses in normal Swiss mice. *Chinese Journal of Immunology*, 4, 176-178 (1988) (in Chinese).

b. Invited Peer-reviewed Review Articles

Hu, C.D. *, Choo, R., and Huang, J. Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front Oncol*, Apr 14;5:90. Doi: 10.3389/fonc.2015.00090 (2015)

Kodama, Y. and Hu, C.D.* Bimolecular fluorescence complementation (BiFC): A 5-year update and future perspectives. *Biotechniques*, 53:285-298 (2012)

Shyu, Y. and Hu, C.D.* Recent advances in fluorescence complementation-based technologies. *Trends Biotechnol.* 26:622-630 (2008)

Hu, C.D.*, Zhang, X.-H., and Bi, E.-H. Role of macrophages in the modulation of NK activity. *Foreign Medicine, Part of Immunology*, 10, 16-20 (1987) (in Chinese).

c. Invited Review Article (Not peer-reviewed)

Shyu, Y., Akasaka, K., and Hu, C.D.*. Bimolecular fluorescence complementation (BiFC): A colorful future in drug discovery. Sterling-Hoffman Life Science Journal, July, 2007. (<http://www.sterlinglifesciences.com/newsletter/articles/article006.html>).

d. Book Chapters

Pratt, E.P.S., Owens, J.L., Hockerman, G.H., and Hu, C.D. Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interactions and assessment of subcellular localization in live cells. High resolution imaging of proteins in tissues and cells: light and electron microscopy methods and protocols (Ed, Schwartzbach, S.D., Skalli, O., and Schikorski, T.), Springer (2015).

Ejendal, K.F.K., Conley, J.M., Hu, C.D. and Watts, V.J. Bimolecular fluorescence complementation analysis of G protein-coupled receptor dimerization in living cells. *Methods Enzymol.*, 521:259-279 (2013).

Kodama, Y. and Hu, C.D.* Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interaction: How to calculate signal-to-noise ratio. *Methods Cell Biol.*, 113: 107-121 (2013).

Vidi, P.A., Przybyla, J., Hu, C.D., and Watts, V.J. Visualization of G protein-coupled receptor (GPCR) interactions in living cells using bimolecular fluorescence complementation (BiFC). *Curr. Protoc. Neurosci.*, Unit 5.29.1-5.29.15 April 2010.

Hu, C.D., Grinberg, A.V. and Kerppola, T.K. Visualization of Protein Interactions in Living Cells Using Bimolecular Fluorescence Complementation (BiFC) Analysis. (ed. Coligan JE, Dunn BM, Speicher DW, Wingfield PT) *Curr. Protoc. Protein Sci.* 41:19.10.1-19.10.21. Hoboken, John Wiley & Sons, 2005.

Hu, C.D. and Kerppola TK. Direct visualization of protein interactions in living cells using bimolecular fluorescence complementation analysis. *Protein-Protein Interactions* (ed. P. Adams and E. Golemis), Cold Spring Harbor Laboratory Press. Pp673-693, 2005.

Hu, C.D., Grinberg A., and Kerppola TK. Visualization of protein interaction in living cells using bimolecular fluorescence complementation (BiFC) analysis. In *Curr. Protoc. Cell Biol.* (ed. Bonifacino JS, Dasso M, Harford JB, Lippincott-Schwartz J, Yamada KM) pp. 21.3.1-21.3.21. Hoboken, John Wiley & Sons, 2005

Kataoka, T., Kariya, K., Yamawaki-Kataoka, Y., Hu, C.D., Shirouzu, M., Yokoyama, S., Okada, T., and Shima, F. Isoprenylation-dependent and independent interaction of Ras with its effectors. In Kuzumaki, N. Cytoskeleton and G-Protein in the Regulation of Cancer. *Hokaido University Medical Library Series*, 37, 141-146 (1998).

Current and Past Grant Support at Purdue University as PI or Co-PI (2003-2017):

Active Grant Support

Title: Role and targeting of PRMT5 in prostate cancer

Source: NCI RO1

Role: Contact PI (**Multi-PI** with Chenglong Li and Jiaoti Huang)

Total Cost Requested: \$2,590,428

Grant Period: 06/09/2017-05/31/2022

Goal: The goal of this proposal is to elucidate the molecular mechanisms by which PRMT5 promotes prostate cancer cell growth, improve the potency of BLL3.3, and conduct a preclinical evaluation of PRMT5 inhibition for castration resistant prostate cancer treatment.

Title: Co-targeting of androgen synthesis and androgen receptor expression as a novel treatment for castration resistant prostate cancer

Source: DoD (2015 PCRP)

Role: PI

Grant Period: 08/01/16-07/30/19

Total Cost: \$557,000

Goal: The goal of this project is to evaluate whether co-targeting of androgen synthesis by abiraterone and androgen receptor expression via PRMT5 inhibition is an effective treatment for CRPC.

Title: Targeting neuroendocrine differentiation for prostate cancer radiosensitization

Source: DoD (2012 PCRP)

Grant Period: 09/30/13-09/30/17

Total Cost: \$559,055

Role: PI

Goal: The goal of this grant is to use CREB targeting as a model to determine whether targeting radiation-induced NED can be explored as a novel radiosensitization approach for prostate cancer radiotherapy.

Title: Developing novel therapeutic strategies for castration-resistant prostate cancer

Source: DOoD (2013 PCRP)

Total Cost: \$525,568

Role: Co-PI (PI: Kavita Shah)

Grant Period: 08/01/14-07/30/18 (no cost extension for current year)

Goal: The goal of this project is to determine whether targeting LIMK2 can be used to treat CRPC.

Title: Development of novel small molecule inhibitors targeting protein arginine methyltransferase 5

Source: CTSI (Indiana Drug Discovery Alliance)

Period: 12/01/14-12/30/17 (No cost extension for current year)

Total amount awarded: \$10,000

Role: PI

Goal: The goal of this project is to discover inhibitors for disruption of PRMT5/MEP50 interaction using BiFC-based screening.

Title: Discovery of novel therapeutic targets for neuroendocrine prostate cancer

Source: Department of MCMP Research Enhancement Award, Purdue University
Period: 04/01/17-03/30/18
Total amount awarded: \$50,000
Role: PI
Goal: The goal of this award is to discovery altered ion channels in neuroendocrine prostate cancer as therapeutic targets

Past Grant Support at Purdue University (2003-2016):

External Funding

Title: Temporal and spatial interaction patterns of bZIP proteins in living *C. elegans*
Source: National Science Foundation (MCB 0420634)
Role: PI
Grant Period: 08/15/04 – 07/30/08
Total Cost: \$ 458,000
Goals: The goal of this project was to establish *C. elegans* BiFC assay to visualize temporal and spatial interactions of *C. elegans* bZIP proteins.

Title: Temporal and spatial interaction patterns of bZIP proteins in living *C. elegans*
Source: National Science Foundation (MCB 0420634)
Role: PI
Grant Period: 06/04/07 – 07/30/08
Total Cost: \$4,750
Goals: The goal of this REU was to support Summer High School Student Research on the funded NSF *C. elegans* project.

Title: Regulation of *c-jun* transcription by ATF2 in cardiomyocyte in response to stress
Source: American Heart Association (AHA 0655570Z)
Role: PI
Grant Period: 07/01/06 – 06/30/08
Total Cost: \$132,000
Goals: The goal of this project was to study the role of ATF2 subcellular localization in regulating *c-jun* transcription in rat cardiomyocytes in response to hypoxia and oxidative stress.

Title: Interplay of CREB and ATF2 in radiation-induced prostate cancer transdifferentiation
Source: DoD Prostate Cancer Idea Development Award (PC073981)
Role: PI
Grant Period: 06/01/08-05/30/11
Total Cost: \$571,875

Goals: The goal of this project was to determine how CREB and ATF2 oppose each other at the transcriptional level to regulate radiation-induced neuroendocrine differentiation in prostate cancer cells.

Title: Improvement of BiFC technology and its application in the TLR signal transduction pathway (International collaborative project)

Source: Natural Science Foundation of China

Role: PI

Grant Period: 01/01/11-12/31/13

Total Cost: \$35,000

Goal: The goal of this project was to collaborate with Dr. Yayi Hou at Nanjing University to apply BiFC technologies to study the TLR signaling in immune system.

Title: D2 receptor-induced sensitization of adenylate cyclase

Source: NIH RO1 (National Institute of Mental Health)

Role: Co-Investigator (PI: Val Watts)

Grant Period: 08/15/11-04/31/14

Total Cost: \$770,922

Goal: The goal of this RO1 grant was to investigate the molecular mechanisms underlying D2 receptor-induced sensitization of adenylate cyclase. As a Co-Investigator, Dr. Hu provided his expertise in BiFC technology to help the analysis of D2 receptor interacting proteins.

Title: New mechanism for modulating opioid receptor mediated analgesia

Source: Showalter Trust Award

Role: Co-PI (PI: Richard van Rijn)

Total Cost: \$75,000

Grant Period: 07/01/14-06/30/16

Goal: The goal of the project is to study the mechanisms and regulation of opioid receptors and to develop agents targeting protein-protein interactions using BiFC-based technologies.

Title: Targeting PRMT5 as a novel radiosensitization approach for primary and recurrent prostate cancer radiotherapy

Source: DoD (2011 PCRP)

Role: PI

Grant Period: 08/01/12-07/30/16

Total Cost: \$559,269.91

Goal: The goal of this grant is to determine that PRMT5 is a novel therapeutic target for prostate cancer radiotherapy.

Title: Identification of the Ac5 sensitization interactome using BiFC

Source: NIH R21 (National Institute of Mental Health)

Role: Multi-PI with Val Watts

Total Cost: \$463,111

Role: Multi-PI

Grant Period: 07/19/13-06/15/17

Goal: The goal of this project is to develop BiFC-based cDNA library screening for identification of Ac5 interacting proteins.

Internal Funding

Title: PRMT5 in prostate cancer development, progression and therapy response

Source: EVPRP Targeted RO1

Period: 12/01/15-05/30/17

Total amount awarded: \$30,000

Role: PI

Goals: The goal of this project is to generate genetically modified mouse models (PRMT5 transgenic mice and PRMT5 Floxed mice) for prostate cancer research.

Title: Discovery of PRMT5 target genes in neuroendocrine prostate cancer

Source: Purdue University Center for Cancer Research

Period: 12/01/16-06/30/17

Total amount awarded: \$10,000

Role: PI

Goals: The goal of this grant is to perform RNA-seq and ChIP-seq to identify target genes of PRMT5 contributing to the development of neuroendocrine prostate cancer.

Title: Mass spectrometric identification of pCREB interacting proteins in prostate cancer cells LNCaP

Source: Purdue Cancer Center Small Grant (Indiana Elks, Inc)

Role: PI

Grant Period: 03/01/08-02/28/09

Total Cost: \$10,000

Goals: The goal of this project was to identify cytoplasmic interacting proteins of pCREB using mass spectrometry.

Title: Identification of interacting proteins and phosphorylation of ATF2 implicated in prostate cancer transdifferentiation

Source: Purdue Research Foundation

Role: PI

Grant Period: 06/01/08-05/30/09

Total Cost: \$16,835

Goals: The goal of this PRF support was to use mass spectrometry to identify interacting proteins and phosphorylation of ATF2 in the cytoplasm in radiation-induced neuroendocrine cells and to determine how ATF2 nuclear import is impaired by ionizing radiation.

Title: Targeting of prostate cancer transdifferentiation and proliferation via a novel DNA nanotube-based nucleic acid delivery

Source: Lilly Seed Grant

Role: PI

Grant Period: 01/01/09-12/31/10

Total cost: \$100,000

Goal: The goal of this grant was to collaborate with Dr. Chengde Mao to develop DNA nanotube-based delivery of siRNAs.

Title: Targeting neuroendocrine differentiation as a novel therapeutics in prostate cancer treatment

Source: Purdue Research Foundation

Role: PI

Grant Period: 08/01/2010-07/30/2011

Total cost: \$17,000

Goal: The goal of this project was to support graduate student Chris Suarez to study the role of radiation-induced neuroendocrine differentiation in radioresistance.

Title: Ionizing radiation induces neuroendocrine differentiation in nude mice prostate cancer xenograft models: Implication in disease progression

Source: Purdue University Center for Cancer Research

Role: PI

Grant Period: 01/01/09-12/31/11

Total Cost: \$50,000

Goals: The goal of this project was to use xenograft nude mice prostate cancer cell models to investigate whether CREB and ATF2 contribute to radiation-induced neuroendocrine differentiation *in vivo* and to determine whether radiation induces changes of pCREB and ATF2 subcellular localization.

Title: Generation of cytoplasmic-localized ATF2 transgenic mice for prostate cancer research

Source: Purdue University Center for Cancer Research

Role: PI

Grant Period: 06/01/10-05/30/11

Total cost: \$2,000

Goal: The goal of this support was to supplement the cost for making a transgenic mouse strain using the shared transgenic mouse facility

Title: Chromogranin A, a novel biomarker to monitor radiation-induced neuroendocrine differentiation in prostate cancer patients

Source: The Indiana Clinical and Translational Science Institute (CTSI)-Purdue Project Development Program

Role: PI

Grant Period: 06/01/10-05/30/12

Total cost: \$10,000

Goal: The goal of this support was to conduct a pilot clinical study to determine the effect of radiotherapy on neuroendocrine differentiation in prostate cancer patients.

Title: Acquisition of an Nikon A1 Confocal Microscope

Source: Lilly Seed Grant, College of Pharmacy

Role: PI

Grant Period: 07/01/11-06/30/12

Total amount awarded: \$300,000

Goal: The goal of this support was to acquire Nikon A1 confocal microscope to set up a Pharmacy Live Cell Imaging Facility

Title: Ultrahigh performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry

Source: Office of the Vice President for Research (OVPR) Laboratory Equipment Program

Role: Co-PI (PI: Andy Tao)

Period: Purchased by May 31, 2014

Total amount awarded: \$100,000

Goal: The goal of this internal support was to acquire UHPLC.

Title: Generation of PRMT5 transgenic mice for prostate cancer research

Source: Purdue University Center for Cancer Research Shared Resource Grant

Period: 12/01/15-12/31/16

Total amount awarded: \$3,100

Role: PI

Goal: The goal of this project is to use the transgenic mouse facility to generate PRMT5-overexpressing mice.

Past Grant Support at Kobe University as PI (1998-2001): \$80,000

Title: Regulation of Rap1A activity by phosphorylation

Source: Kobe University, President Young Investigator Award

Role: PI

Grant Period: 04/01/98-03/30/99

Total Cost: ~\$10,000 (for supplies)

Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to antagonize the function of Ras in activating Raf-1.

Title: Effect of phosphorylation on the regulation of Rap1A activity

Source: Ministry of Education, Science, Sports, and Culture of Japan

Role: PI

Grant Period: 04/1/98 - 03/30/99

Total Cost: ~\$ 10,000 (for supplies)

Goals: The goal of this project was to investigate whether phosphorylation of Rap1A by PKA affects the ability of Rap1A to activate downstream effectors such as Raf-1 and B-Raf.

Title: Activation mechanism of phospholipase C (PLC- ϵ) by Ras

Source: Hyogo Science and Technology Association

Role: PI

Grant Period: 04/01/00 – 03/30/01

Total Cost: ~\$ 30,000 (for supplies)

Goals: The goal of this project was to investigate whether Ras regulates catalytic activity of PLC ϵ directly by their physical interaction. The approach was to use *in vitro* reconstitution system.

Title: Regulation of a novel phospholipase C (PLC- ϵ) by Ras

Source: Japan Society for the Promotion of Science

Role: PI

Grant Period: 04/01/00 – 03/30/01

Total Cost: ~\$ 30,000 (for supplies)

Goals: The goal of this project was to investigate how Ras regulates catalytic activity of PLC ϵ and determine whether membrane anchoring of PLC- ϵ by Ras is sufficient for the activation of PLC- ϵ . This project was primarily focused on the studies in cells.

Note: Research grants in Japan do not provide personnel support. All faculty members and staff are supported by the government. Postdoctoral fellows and graduate students can only be supported by fellowships.

Fellowships/Awards received by trainees

- Susan Fox, Ross Fellowship (08/2003-07/2005): ~\$56,000
- Susan Fox, 2nd place of graduate student presentation
2004 Walther Cancer Institute Annual Retreat (Aug. 5-7)
- John Y Shyu, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) (\$866)
- Susan Fox, graduate student, Travel Award from 15th International Worm Meeting (June 25-29, 2005, Los Angeles) (\$866)
- Zeina Shtaih, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program), \$4,000
- Jonathan Smith, Pharmacy Student, Summer Research Fellowship (2005 Breast Cancer Research Program), \$2,000
- Jonathan Smith, NSF, Summer Research Fellowship (REU), \$6,000 (IC \$1,000)
- Apinya Supatkul, Prepharmacy Student, 2006 Summer Research Fellowship (\$3,000)
- John Shyu, 1st Place of 2007 Purdue University Graduate Student Research Competition (\$500)
- Holli Duren, Travel Award from 16th International Worm Meeting (June 27-July 1, 2007, UCLA) (\$300)
- John Shyu, John Koo Travel Award for Fall 2007 (\$1,000)

- Holli Duren, Kienly Award for outstanding graduate student teaching assistant 2007, MCMP (\$750)
- Holli Duren, 2007 PRF Summer Fellowship (\$2,472.09)
- Holli Duren, 2008-2009 PRF Fellowship (\$16,835)
- Chris Suarez, Purdue University Doctoral Fellowship (08/2007-07/2009): ~\$56,000
- Susan Fox, Bilsland Dissertation Fellowship (07/2008-12/2008): ~\$14,000
- John Shyu, Bilsland Dissertation Fellowship (07/2008-12/2008): ~\$14,000
- Holli Duren, 2008-2009 Graduate Student Award for Outstanding Teaching at Purdue University
- Holli Duren, 2009 Charles J. Paget Travel Award: \$1,000
- Yutaka Kodama, 04/01/09-03/31/10 TOYOBO Postdoctoral Fellowship (~\$34,000)
- Akhil Shenoy (Texas AM U) , 06/01/09-07/26/09, Purdue SROP: \$5,000
- Yutaka Kodama, 04/01/10-03/31/12, JSPS Postdoctoral Fellowship (~\$80,000)
- Holli Duren, Bilsland Dissertation Fellowship (01/01/2010-06/30/2010): \$14,000
- Chih-chao Hsu, Ronald W. Dollens Graduate Scholarship in Life Sciences (08/2010-05/2011): \$5,000
- Yeo Jin Choi, Purdue University College of Pharmacy 2010 Summer Undergraduate Research Fellowship: \$3,000
- Chris Suarez, 2010 PRF Fellowship: \$17,000
- Chih-chao Hsu, Travel Award for conference attendance from PULSe, \$250 (2012)
- Chih-chso Hsu, 2011 PRF Fellowship: \$17,000
- Chris Suarez, 2011 Paget Travel Award from MCMP department, \$1,000
- Chris Suarez, 2012 AACR Minority Scholar in Cancer Research Award for participation in the Advances in Prostate Cancer Research conference (Feb 6-9, 2012), \$1,800
- Chih-chao Hsu, Bilsland Dissertation Fellowship (09/01/12-12/31/12): \$14,000
- Huanxin Zhang (visiting student from Jinan University, China): Graduate Student Study Abroad Scholarship: \$9,000 (2012)
- Huanxin Zhang (visiting student from Jinan University, China): China Scholarship Council (CSC): \$33,600 (awarded for two years 10/2013-9/2015, but stay for one year)
- Limin Zhang (PharmD student): 2014 Summer Undergraduate Research Fellowship (Lilly Endowment Fellowship): \$4,800
- Jake Owens, Ross Graduate Fellowship (2014-2015), \$38,000
- Athena He: 2016 LSAMP Summer Undergraduate Research Fellowship: \$4,800
- Jonathan Malola: 2017 College of Pharmacy Summer Undergraduate Research Fellowship: \$4,800

- Jake Owens, CTSI Predoctoral fellowship (07/01/17-06/30/19): \$24,500/year plus tuition remission
- Jake Owens, 2nd place of Presentation Award at the 2017 Indiana Urological Research Symposium: \$500

Teaching Experience

Lectures and labs

5/1985-6/1987: Microbiology and Immunology labs (medical students)

7/1987-8/1991: Epidemiology lectures and labs in the Department of Epidemiology, School of Public Health, Tongji Medical University, Wuhan

4/1997-8/2000: Physiology and Molecular Biology lab (medical students) in the Department of Physiology II, Kobe University

8/2003-present: As a faculty member in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University College of Pharmacy, I have been involved in the teaching of the following courses. The class size for the courses ranges from 5~15 for graduate students, 30-40 for BSPS students, and 150 ~205 for professional pharmacy students. The total number of lecture hours taught is approximately 40h/year. Teaching evaluation scores have been 4.5~4.8/5.0. In April 2017, I received the first teaching award of the Pharmaceutical Sciences Teacher of the Year, which was completely nominated and voted by BSPS graduates in the College of Pharmacy.

Courses Taught

Professional Pharmacy Students:

MCMP 305 (Biochemistry I, 2004-2006)

MCMP 304 (Biochemistry II, 2005-2008)

MCMP 440 (Pathophysiology, 2006-2012)

PHRM 824 (Principles of Pathophysiology and Drug Action, 2012-present)

PHRM 302 (Integrated Lab, Neoplasia module, 2005-2012)

PHRM 820 (Professional Program Laboratory, Neoplasia module, 2012-2015)

Graduate students:

MCMP 618/690G (Molecular Targets of Cancer, 2007-present)

MCMP 617/690N (Molecular Targets of Neurological Disorders, 2007-present)

MCMP 514 (Biomolecular Interactions-Theory and Practice, 2009-present)

MCMP 696 (Seminars in Medicinal Chemistry and Molecular Pharmacology, 2006-2008)
MCMP 599 (Cumulative written examinations, 2015-present)

Undergraduate students (BS in Pharmaceutic Sciences):
PHRM 460 (Drug Discovery and Development I, 2013-present)
MCMP 544 (Drug Classes and Mechanisms, 2015-present)

Medical students (Indiana School of Medicine):
LCME 504 (Molecular Cell Biology, guest lecture of Molecular Biology of Cancer, 2013-2015)

Courses Served as Coordinator

PHRM 824 (Principles of Pathophysiology and Drug Action, 2013-present)
MCMP 440 (Pathophysiology, 2011-2012)
MCMP 696 (Seminars in Medicinal Chemistry and Molecular Pharmacology, 2006-2008)
MCMP 599 (Cumulative written examinations, 2015-present)

Supervision of graduate, professional and undergraduate student research

07/1987-08/1991 Supervised 6 undergraduate students at Tongji Medical University
04/1997-08/2000 Co-supervised 7 Ph.D. students for thesis research with Professor Tohru Kataoka and supervised 5 undergraduate summer research at Kobe University.
09/2000-06/2003 Supervised two undergraduate students at University of Michigan
07/2003-present (1) Served as thesis adviser of 8 Ph.D. students (6 graduated) and 2 master students (graduated) and co-adviser of 5 Ph.D. students (4 graduated)
 (2) Served as a thesis committee member of 45 graduate students
 (3) Served as a committee member of 36 oral preliminary examination
 (4) Supervised 39 graduate students for lab rotations
 (5) Supervised 31 professional and undergraduate student research
 (6) Supervised 4 high school students for summer research

Supervision of postdoctoral fellows, visiting scholars and technicians

07/2003-present Supervised 9 postdoctoral fellows, visiting scholars and technicians

Service Experience

Major Administrative Services in the Purdue University Center for Cancer Research

2010-2013 **Seminar Coordinator** of Purdue University Center for Cancer Research

2010-Present **Co-leader** of Prostate Cancer Discovery Group of Purdue University Center for Cancer Research

2012- Present **Coordinator** of Indian Basic Urological Research (IBUR) monthly meetings

2012- Present **Executive Committee Member** of Obesity and Cancer Discovery Group, Purdue University Center for Cancer Research

2013- Present **Executive Member** of Purdue University Center for Cancer Research

2013- Present **Co-leader**, Cell Identity and Signaling (CIS) Program of Purdue University Center for Cancer Research

Major Administrative Services at Purdue University

2007-2009 PULSe Graduate Program Admission Committee

2007-2009 PULSe Graduate Program Recruitment Committee

2008-present Bindley Imaging Committee (BIG)

2010 Faculty Search Committee for a Cancer biology and Pharmacology position in the College of Veterinary Medicine

2012-present PULSe Graduate Program Curriculum Committee

Major Administrative Services in the College of Pharmacy

2009-2013 Assessment Committee

2011-present Director of Pharmacy Live Cell Imaging Facility (PLCIF)

2011-present Chair of PLCIF Committee

2012-2014 Grade Appeal Committee

2012-present Faculty Liaison for Core-Pharmacy Courses Taught by Other Schools (BIOL110/111)

2013-2014 Honor Degree Policy Committee

2013-2016 Curriculum committee

2014-present Pharm.D. Academic Standards and Readmissions Committee

Major Administrative Services in the Department of Medicinal Chemistry and Molecular Pharmacology

2005-2011 Facility and Instrumentation Committee

2008-2009 Strategy Plan Task Force

2009 Biochemistry Task Force

2010 Business Manger Search Committee

2011 Faculty Search Committee (Pharmacology)

2012 Faculty Search Committee (Pharmacology)

2012 Faculty Search Committee (Epigenetics)

2010-2015 Graduate Admissions and Recruiting Committee
2012-present Graduate Assessment Committee
2015-present Chair of Graduate Assessment Committee
2016 Chair of faculty search committee